PB82-244369

An Ultraviolet-Visible Spectrophotometer Automation System. Part I Functional Specifications

Cincinnati Univ. Computing Center, OH

Prepared for

Environmental Monitoring and Support Lab.
Cincinnati, OH

Jul 82

U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA-600/4-82-053a	ORD Report	PB82 244369

4. TITLE AND SUBTITLE	5. REPORT DATE
"An Ultraviolet-Visible Spectrophotometer Automation System - Part I Functional Specifications"	July 1982
	6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
Gregory Roth, John Teuschler, William Budde	

9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
University of Cincinnati Computing Center Cincinnati, Ohio	AIDBID
	11. CONTRACT/GRANT NO.
	GS-05S-10458

12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
Environmental Monitoring and Support Laboratory U.S. Environmental Protection Agency Office of Research and Development 26 W. St. Clair St., Cinti., OH 45268	Applications Guide 10/79-3/80
	14. SPONSORING AGENCY CODE EPA 600/06

15. SUPPLEMENTARY NOTES

16. ABSTRACT

This document contains the project definition, the functional requirements, and the functional design for a proposed computer automation system for scanning spectrophotometers. The system will be implemented on a Data General computer using the BASIC language.

The system is a real-time data acquisition system with "on-line" data reduction, quality assurance, report generation and plotting. It is designed for operation with the Environmental Protection Agency (EPA) Data General automation system.

Analyses included in the system facilitate the determination of chlorophyll concentrations, the evaluation of color in waters, and a general transmittance/absorbance measurement of any given sample. Quality assurance is provided by the analysis of standard filters.

Two related publications are titled, "An Ultraviolet-Visible Spectrophotometer Automation System - Part II User's Guide" and "An Ultraviolet-Visible Spectrophotometer Automation System - Part III Program Documentation."

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
Release to public	Unclassified	45
	20. SECURITY CLASS (This page) Unclassified	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

EPA 600/4-82-053a
July 1982

PB82-244369

AN ULTRAVIOLET-VISIBLE SPECTROPHOTOMETER AUTOMATION SYSTEM PART I - FUNCTIONAL SPECIFICATIONS

by

Gregory S. Roth
University of Cincinnati Computing Center
Cincinnati, Ohio 45220

John M. Teuschler and William L. Budde
Physical and Chemical Methods Branch
Environmental Monitoring and Support Laboratory
Cincinnati, Ohio 45268

Contract No. GS-05S-10458

ENVIRONMENTAL MONITORING AND SUPPORT LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

NOTICE

Mention of trade names or commercial products does not consititute endorsement or recommendation for use.

FOREWORD

Environmental measurements are required to determine the quality of ambient waters and the character of waste effluents. The Environmental Monitoring and Support Laboratory - Cincinnati, conducts research to:

- Develop and evaluate techniques to measure the presence and concentration of physical, chemical, and radiological pollutants in water, wastewater, bottom sediments, and solid waste.
- Investigate methods for the concentration, recovery, and identification of viruses, bacteria and other microbiological organisms in water; and to determine the responses of aquatic organisms to water quality.
- Develop and operate an Agency-wide quality assurance program to assure standardization and quality control of systems for monitoring water and wastewater.
- Develop and operate a computerized system for instrument automation leading to improved data collection, analysis, and quality control.

This report was developed in the Advanced Instrumentation Section of the Environmental Monitoring and Support Laboratory in the interest of advancing laboratory techniques and quality control through computerization.

Robert L. Booth

Robert L. Booth, Acting Director
Environmental Monitoring and Support
Laboratory - Cincinnati

BEGIN FIRST LINE OF TEXT HERE
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD; BEGIN SECTIONS HERE

ABSTRACT

This document contains the project definition, the functional requirements, and the functional design for a proposed computer automation system for scanning spectrophotometers. The system will be implemented on a Data General computer using the BASIC language.

The system is a real-time data acquisition system with "on-line" data reduction, quality assurance, report generation and plotting. It is designed for operation with the Environmental Protection Agency (EPA) Data General automation system.[1]

Analyses included in the system facilitate the determination of chlorophyll concentrations, the evaluation of color in waters, and a general transmittance/absorbance measurement of any given sample. Quality assurance is provided by the analysis of standard filters.

Two related publications are titled "An Ultraviolet-Visible Spectrophotometer Automation System - Part II User's Guide" and "An Ultraviolet-Visible Spectrophotometer Automation System - Part III Program Documentation."

This report was submitted in partial fulfillment of Task Assignment Nos. EPA-79-219 and EPA-80-07, of EPA Contract No. GS-05S-10458 by the University of Cincinnati Computer Center. The report covers work done between October 10, 1979, and February 26, 1980, and work was completed as of February 1980.

BEGIN LAST LINE OF TEXT
EPA Form 2340-4 (1-80)
TYPING GUIDE SHEET

CONTENTS

Foreword..... iii
Abstract..... iv
Figures..... vii
Tables..... viii
Acknowledgments..... ix

1. Introduction..... 1
2. Project Definition..... 2
3. Functional Requirements - Overview of the Project..... 3
   3.1 Scientific principals of spectrophotometric analysis..... 3
   3.2 Instruments to be automated..... 3
   3.3 Non-technical considerations..... 4
      3.3.1 Time and financial considerations..... 4
      3.3.2 Existing hardware and software to be used..... 4
      3.3.3 Support of the completed system..... 5
   3.4 Future considerations..... 6
4. Functional Requirements - Requirements of the System..... 7
   4.1 Raw data acquisition..... 7
   4.2 Wavelength calibration and scan speed..... 7
      4.2.1 Wavelength calibration procedure..... 7
      4.2.2 Wavelength calibration checking procedure..... 8
      4.2.3 Scan speed..... 8
   4.3 Transmittance calibration..... 8
   4.4 Spectrophotometer quality assurance - filter test..... 9
      4.4.1 Data acquisition and reduction..... 9
      4.4.2 The filter tests..... 9
      4.4.3 Historical control standards..... 10
      4.4.4 Precision and accuracy evaluation..... 10
         Tristimulus integrator performance..... 10
         Transmittance measurement performance..... 11
      4.4.5 Report requirements..... 11
         Filter scan reports..... 11
         Summary report..... 11
   4.5 Chlorophyll analysis..... 11
      4.5.1 Existing method..... 11
      4.5.2 Data acquisition and reduction..... 12
         Automated method..... 12
         Manual entry alternative method..... 12
      4.5.3 Peak absorbance choice..... 14
      4.5.4 Replicate statistics..... 14
      4.5.5 Report and plot requirements..... 14
         Sample and summary reports..... 14

Sample plot..... 15
4.5.6 Sample file control interaction..... 15
4.6 Color analysis..... 15
4.6.1 Existing method..... 15
4.6.2 Data acquisition and reduction..... 16
Automated method..... 16
Manual entry alternative method..... 17
4.6.3 Standards and quality control..... 17
4.6.4 Report and plot requirements..... 17
Intermediate standards reports..... 17
Intermediate unknowns report..... 17
Summary standards report..... 18
General summary report..... 18
Plots..... 18
4.7 Sample transmittance/absorbance analysis..... 18
4.7.1 Data acquisition and reduction..... 18
4.7.2 Report and plot requirements..... 19
Sample report..... 19
Summary report..... 19
Sample plots..... 19
5. Functional Design..... 20
5.1 General description of the UVVIS system..... 20
5.1.1 Master control level..... 20
5.1.2 Set-up and instrument checkout..... 20
5.2 General design of the analysis modules..... 21
5.3 Design of calibration setting and checking procedures..... 21
5.3.1 Wavelength calibration..... 21
Checking calibration..... 23
5.3.2 Scan speed..... 23
Checking scan speed..... 23
5.3.3 Transmittance calibration..... 23
Checking calibration..... 24
5.4 Optional quality assurance test..... 24
5.4.1 Description of an automated run..... 24
5.4.2 Example reports..... 25
5.4.3 Data files..... 25
5.5 Optional chlorophyll analysis..... 25
5.5.1 Description of an automated run..... 25
5.5.2 Description of a manual run..... 30
5.5.3 Example reports and plots..... 30
5.5.4 Data files..... 30
5.6 Optional color analysis..... 30
5.6.1 Description of an automated run..... 30
5.6.2 Description of a manual run..... 34
5.6.3 Example reports and plots..... 34
5.6.4 Data files..... 34
5.7 Plotting and the plot reference file..... 40
6. Signoff sheet..... 41

References..... 42
Appendix A. Three-filter tristimulus method of manual color analysis..... 44

FIGURES

Number		Page
1	General scheme of the UVVIS system	22
2	General design of the UVVIS analysis modules	22
3	Intermediate quality assurance report format for an NBS tristimulus filter	26
4	Intermediate quality assurance report format for an NBS linearity filter	27
5	Summary report format for quality assurance analysis	28
6	Automated chlorophyll analysis command structure	29
7	Intermediate chlorophyll report format for a sample	31
8	Example chlorophyll plot of a sample	32
9	Summary report format for chlorophyll analysis	32
10	Features and command structure of the automated color analysis	33
11	Intermediate color report format for update standards	35
12	Intermediate color report format for check standards	35
13	Intermediate color report format for unknowns	36
14	Summary standards report format for color analysis	36
15	General summary report format for color analysis	37
16	Example color plot of an update standard	38
17	Example color plot of a check standard	38
18	Example color plot of an unknown	39
19	An example of the superimposed grid	39

TABLES

Number		Page
1	NBS certified tristimulus filter values (Set 137).....	9
2	NBS certified linearity filter value.....	10
3	Wavelengths used in the trichromatic computational methods.....	12
4	Equations used in the trichromatic computational methods.....	13
5	Transmittance calibration wavelength ranges.....	24
6	Filters used by the three-filter tristimulus method.....	45

ACKNOWLEDGMENTS

A number of people contributed to software related specifications for this system. Dr. Cornelius Weber of the Environmental Monitoring and Support Laboratory - Cincinnati provided requirements for the proper methodology of chlorophyll analysis, as well as a FORTRAN IV program for chlorophyll computation and report generation. Larry Lobring, also of the Environmental Monitoring and Support Laboratory - Cincinnati assisted in the development of color analysis and quality assurance requirements. Dennis Ryan of the University of Cincinnati Computing Center made all major developments of the Ultraviolet-Visible Spectrophotometer (UVVIS) automation system, other than sample transmittance/absorbance analysis. His efforts included insightful design of data reduction algorithms for color analysis and quality assurance.

Special appreciation is extended to Dr. George Garland of the DuPont Company, who supplied several industrial dye samples and test results. These contributions were useful in the design of the color analysis module.

SECTION 1

INTRODUCTION

These functional specifications describe an automation package for scanning spectrophotometers. The report is divided into four main areas: project definition, functional requirements-overview of the project, functional requirements-requirements of the system, and functional design. The combination of these four areas describes the system so as to enable an operator to decide if the specified system will meet his requirements.

The project definition consists of the scope of the project as arrived at among users, implementors, and management. It contains a brief written statement of the overall goals of the project.

The "functional requirements-overview of the project" section contains a brief description of spectrophotometric principles, a description of the existing system and instrumentation, and any technical constraints imposed on the proposed system. The support issue is also addressed under the non-technical considerations in order to inform management of the additional manpower requirements or reliance on resources outside the laboratory in which the instrument was automated.

The "functional requirements-requirements of the system" section contains a detailed list of the requirements of the automation system. This list includes hardware requirements, calculation requirements, reporting requirements, and all interaction necessary with the system to obtain the desired objectives in the project definition. Enough information must be contained in the functional requirements to allow a functional design to be formulated. The functional design is a "blueprint" of the system, showing a flowchart of the order of operations, and a detailed description of all input, output, and human interaction. The functional requirements are used to produce a design framework linking all the functional blocks. The functional design is then used in the implementation phase which entails producing the detailed design, building the appropriate hardware and software modules, and testing the system components.

The final system will provide full documentation of all hardware and software modules and a users' manual for "operator-automation system" interaction.

These functional requirements were written to satisfy the needs of Region III, Region V, and the Environmental Monitoring and Support Laboratory, Cincinnati (EMSL-Cincinnati).

SECTION 2

PROJECT DEFINITION

The purpose of this project is to develop a computer system which will automate the various analyses performed on a scanning ultraviolet/visible spectrophotometer. The system must be developed in such a fashion as to allow a variety of commercially available scanning spectrophotometers to be interfaced to the system. The system will provide:

- optional analysis of chlorophyll and pheophytin a concentrations in environmental samples;
- optional analysis of color levels in environmental samples;
- optional filter testing to evaluate spectrophotometer as a color tristimulus integrator or as a transmittance/absorbance measuring device;
- optional scanning of various samples to determine transmittance over specified wavelength range;
- provisions for detailed reports on various computer output devices;
- plotting capability on Tektronix terminals or similiar plotting terminals which are software compatible;
- wavelength encoding with ±0.1 nm. resolution in precision of recorded wavelength values;
- a transmittance measurement capability of one part in 10,000;
- computer control of the scanning mechanism; and
- a fully automated or semi-automated mode of operation.

SECTION 3

FUNCTIONAL REQUIREMENTS - OVERVIEW OF THE PROJECT

3.1 SCIENTIFIC PRINCIPLES OF SPECTROPHOTOMETRIC ANALYSIS

A spectrophotometer measures the intensity of light or radiant energy which is transmitted through a solution or solid at various wavelengths. Normal daylight is composed of many wavelengths of light. The spectrophotometer is designed to send a nearly single-wavelength (monochromatic) beam of light through a sample.

Most spectrophotometers operate in the wavelength ranges of visible and ultraviolet light. The visible light wavelength range is approximately 370 to 800 nanometers, and a tungsten lamp is used as the light source. The ultraviolet range is about 190 to 370 nanometers, and the source is a deuterium lamp. Any particular wavelength can be set on the instrument. Most spectrophotometers can also scan from one wavelength to another.

In analyzing a sample solution or solid, the nearly monochromatic wavelength beam of light is sent from the source lamp, through the sample, and onto a photodetector. The photodetector measures the amount of light (of a particular wavelength) that has passed through the sample.

Spectrophotometers may be either single-beam or double-beam machines. A single-beam apparatus sends the source beam through the sample to the photodetector. A double-beam instrument splits the source beam into two beams, sending one through the sample and the other through a reference of clear solution or air. The two beams are received separately and a relative intensity is determined. In this way, a double-beam apparatus is able to correct for fluctuations in the intensity of the source beam.

3.2 INSTRUMENTS TO BE AUTOMATED

This version of the system is to be designed in consideration of automating the following instruments: 1) Perkin-Elmer Double Beam Spectrophotometer, Coleman model 124, at the Environmental Monitoring and Support Laboratory - Cincinnati, Ohio; 2) Perkin-Elmer model 356 Spectrophotometer, at the EPA Region V Surveillance and Analysis Laboratory, Chicago, Illinois; and 3) Varian model 635 Spectrophotometer, at the EPA Region III Surveillance and Analysis Laboratory, Annapolis, Maryland.

3.3 NON-TECHNICAL CONSIDERATIONS

3.3.1 Time and Financial Considerations

The Automated Ultraviolet-Visible Spectrophotometer System (UVVIS) is to be operational by June 1980. Manuscript copies of the users' guide and program documentation should be completed by July 1980. Total effort should take less than 0.5 man years. The cost for functional specifications, implementation design and final documentation should not exceed $25,000. This figure includes $15,000 of contractor programming effort and $10,000 of "in-house" engineering effort.

The initial software and hardware development cost may be distributed over additional instruments which are interfaced to the automation system. The software (application code and real-time assembly language) will be directly transportable to other USEPA automation systems. The individual hardware cost to interface an instrument should not exceed $1000 excluding the user terminal. The $1000 includes the interface at the instrument (hardware analog drivers and control circuitry for the scanning mechanism) and the additional hardware necessary at the Data General computer. The cost does not include adapting the mechanical encoder to the particular spectrophotometer. The complexity of this task will vary with the types of spectrophotometers. Travel and training are not included in the above cost.

3.3.2 Existing Hardware and Software to be Used

The UVVIS project is one branch of a much larger laboratory automation project. Therefore, some of the basic decisions about system hardware and software have already been made within the context of the larger project. Thus, these functional requirements are not strictly hardware and software independent.

This project will use an existing Data General NOVA 840 minicomputer with the operating system RDOS (Revision 6.2 or 6.x). The RDOS operating system will be upgraded until the current version of the extended BASIC system is not supported.

The multi-user extended BASIC system is currently frozen at Revision 4.3. This is the result of extensive modifications to the sources. That is, the Data General BASIC has been modified by Lawrence Livermore Laboratories to allow real-time data acquisition and control through calls to assembly language routines. Data reduction, operator control, and report generation are accomplished through the high level language BASIC.

Due to the cost in modifying the BASIC sources and associated software, a move to another version of Data General's BASIC will only occur if the present revision fails to satisfy the needs of the agency.

The NOVA 840 minicomputer was interfaced to a general purpose interface card to provide digital input and output (I/O) control. All inputs and outputs are controlled from the BASIC language. Any instrumental control

will, therefore, be implemented with the existing digital I/O hardware. Likewise, a 14 bit analog to digital (A/D) converter was interfaced to the system for a multiplexed input of up to 32 channels of analog input. This design should also be used for the acquisition of analog data. This should not discourage the input of digital data. Multiplexers have been inserted into the standard digital interface for future expansion to digital types of inputs.

3.3.3 Support of the Completed System

Support of an automated system is divided into software and hardware support. In each of these areas the manufacturers' equipment and software have been left unaltered as much as possible. This condition allows independence from any custom hardware or software packages. The complexity of the automation system, however, does require the need for some custom hardware and software. Therefore, this section describes the responsibilities for the maintenance of the various sections of the total system.

The Data General NOVA computer system, excluding the digitial interface and the analog interface, is the responsibility of the Data General Corporation through a maintenance contract which must be maintained by the laboratory using the system. All custom hardware must be removable so that the Data General service engineers have a clean system to troubleshoot. Data General maintains the peripherals, central processing unit, memory, and so on.

The Data General software, RDOS 6.2 and BASIC Rev. 4.3, is the responsibility of the Data General Corporation. Modifications to the BASIC Rev. 4.3 are the responsibility of USEPA-Cincinnati which is a combined effort of Environmental Monitoring and Support Laboratory - Cincinnati (EMSL-CI) and Computer Services and Systems Division - Cincinnati (CSSD-Cincinnati).

Any custom hardware in the UVVIS automation system supplied by the Advanced Instrumentation Section, EMSL-Cincinnati, will be supported by that same group for a minimum of one year after delivery. Mandatory interfacing procedures for instruments dictated by the Advanced Instrumentation Section require a full set of documentation with any custom interface hardware in addition to a complete set of spare cards. This allows "in house" repair of custom hardware through module swapping.

The BASIC application code provided by USEPA-Cincinnati for the automation of various instruments will be supported by the USEPA-Cincinnati for a minimum of one year after delivery. Programming procedures require complete documentation of the application code and of the users' procedures. It must be stressed that while USEPA-Cincinnati will provide a standard software package to requesting laboratories, it will not support changes in the package which are made external to USEPA-Cincinnati. Training on the use of the automation system will be provided by USEPA-Cincinnati. All custom hardware and software provided by EMSL-Cincinnati will be supported for a minimum of one year after delivery.

3.4 FUTURE CONSIDERATIONS

The hardware and system software of the UVVIS system will be designed to be adaptable to any scanning spectrophotometer. The proposed sample transmittance/absorbance analysis component of the system will be the building block for future specific applications. Such applications could include analyses which use the ultraviolet light source. The system is also designed in a modular fashion to permit linking up with the Sample File Control (SFC) system if this were a future requirement.

BEGIN FIRST LINE OF TEXT HERE
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD: BEGIN SECTIONS HERE

SECTION 4

FUNCTIONAL REQUIREMENTS - REQUIREMENTS OF THE SYSTEM

The following section contains a detailed list of the requirements of the UVVIS automation system. From the described requirements a functional design can be developed.

4.1 RAW DATA ACQUISITION

The system must have the capability to collect transmittance data versus encoded wavelength data within a 100 microsecond time interval. The encoded wavelength data must have a minimum resolution of ±0.1 nanometers. The transmittance data or absorbance data must be made available to the 14 Bit analog-to-digital (A/D) hardware of the automation system without having any adverse affects on the signal within the spectrophotometer.

The automation system must be able to activate the scan circuitry of the spectrophotometer to ensure that a valid data matrix reaches the system via the BASIC language. There is an optional reverse scan which may be used on appropriate scanning spectrophotometers.

4.2 WAVELENGTH CALIBRATION AND SCAN SPEED

An automated analysis has the capability to check the existing wavelength calibration, and to recalibrate the wavelength if necessary.

4.2.1 Wavelength Calibration Procedure

The relationship between wavelength encoder value (in nanometers) and wavelength signal (in A/D counts) is assumed to be quadratic, such that:

$$\text{Wavelength} = A + \left[B \times (\text{counts})\right] + \left[C \times (\text{counts})^2\right]$$

where A, B and C are constant coefficients.

Calibration must be made in the range of 300 to 800 nanometers. Readings are to be taken on at least six settings, each of a different wavelength value. The best second degree fit will be determined using the method of least squares regression.[2] A fitting error will be computed to account for normal human error in setting the wavelength dial. This error is called the "standard error of estimate."

BEGIN LAST LINE OF TEXT
3/8"
BOTTOM OF IMAGE AREA OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS

PAGE NUMBER
EPA Form 2350-4 (4-80)
(PREVIOUSLY CIP, EPA FORM 2xx)
TYPING GUIDE SHEET

After the calibration, the following data will be displayed for the existing and new calibrations: the calibration equation, the standard error of estimate, and the percent relative standard error of estimate. The new data may be accepted or rejected.

4.2.2 Wavelength Calibration Checking Procedure

Any number of points may be checked. If a wavelength measurement as determined by the computer is within plus/minus 0.25 nanometers of the expected value, then the reading will be considered acceptable. A warning will be in effect if the difference between the measurement and the expected value falls in the bounds of plus/minus 0.25 and 0.5 nanometers. If the difference exceeds plus/minus .5 nanometers, then the reading will be considered out of control.

For each wavelength tested, the following data will be displayed at the terminal: expected wavelength (nm.), measured wavelength, difference, standard error of estimate, and control rating.

4.2.3 Scan Speed

A different scan speed will be established for each of the analysis functions. Once established, it will not require resetting, unless desired.

The setting routine will entail making a 10 second scan, and computing the nanometer-per-minute scan rate based on the wavelength calibration equation. The computed speed, to the nearest five nanometers, will be displayed at the terminal.

Checking the scan speed will be done by making a 5 second scan. The expected and measured scan speeds will be displayed at the terminal.

4.3 TRANSMITTANCE CALIBRATION

Baseline transmittance calibration setting or checking may be performed before or during the automated analysis of chlorophyll, color, quality assurance, or sample transmittance/absorbance. A separate set of calibration data will be needed for each type of analysis, since the different types employ various sample references (such as reagent, distilled water, and air).

Both the calibration and checking procedures will require initial meter adjustments for the 0% and 100% transmittance levels. The adjustments will be made at the midpoint of the scanning range of the particular analysis.

Calibration will be performed at both 0% and 100% transmittance. Data will be acquired and reduced to transmittance signal values at nanometer-by-nanometer increments. Any percent transmittance (PT) at some wavelength (W) can be determined by the following interpolation equation:

$$PT(W) = \left(\left[T(W) - T'(W) \right] \Big/ \left[T''(W) - T'(W) \right] \right) \times 100,$$

where T(W) is the measured transmittance signal at wavelength W, T'(W) is the 0% transmittance signal at W, and T"(W) is the 100% transmittance signal at W. It should be noted that no special reports will be required for this calibration or check procedure.

4.4 SPECTROPHOTOMETER QUALITY ASSURANCE - FILTER TEST

The precision and accuracy of the automated spectrophotometer will be verified by the use of two types of filter tests. One test will evaluate the automated system as a tristimulus integrator. The other test will determine the automated instrument's ability to measure transmittance.

4.4.1 Data Acquisition and Reduction

Before the filter tests begin, the wavelength calibration may be checked. Transmittance may be calibrated at 0% and 100% levels.

Filter data will be acquired by scanning from 700 to 400 nanometers. These data will be reduced to percent transmittance values at integer nanometer increments from 700 to 400 nanometers.

For the National Bureau of Standards (NBS) Tristimulus filters, the data will be converted to tristimulus values X, Y, and Z. For the NBS Linearity filters, the data will be used to compute the percent transmittance at the wavelengths specified in the NBS certification data.

4.4.2 The Filter Tests

The automated spectophotometer's performance as a tristimulus integrator will be evaluated by testing NBS Tristimulus filters 2101, 2102, 2103, 2104, and 2105.[3,4] The tristimulus values X, Y, and Z computed from scans of these filters will be compared to certified NBS tristimulus values and to historical control data. Table 1 shows the certified tristimulus values for filter set 137.

TABLE 1. NBS CERTIFIED TRISTIMULUS FILTER VALUES (SET 137)

Filter Number	Tristimulus Values X	Y	Z
2101	45.0	25.3	0.0
2102	51.5	48.9	5.6
2103	3.6	11.3	2.6
2104	17.2	9.1	84.3
2105	51.8	56.1	75.4

The transmittance measuring capability of the automated spectrophotometer will be determined by testing NBS Linearity filters 1-282, 2-282, and 3-282 (SRM 930b). The transmittance values at the designated NBS wavelengths will be compared to the NBS certified values (shown in Table 2), and to historical control data.

TABLE 2. NBS CERTIFIED LINEARITY FILTER VALUES

Filter Number	Percent Transmittance at Specified Wavelengths (nanometers) 440.0	465.0	546.1	590.0	635.0
1-282	9.15	10.86	9.78	8.68	9.70
2-282	18.90	21.26	19.78	18.22	19.67
3-282	29.16	32.27	30.73	27.71	27.83

4.4.3 Historical Control Standards

Historical data will be maintained for control standards. These data will be used in spectrophotometer precision evaluation. As many as 25 sets of control data for each filter will be maintained. For NBS Tristimulus filters, individual tristimulus values X, Y, and Z will be stored, in addition to means and standard deviations for each X, Y, and Z. For NBS Linearity filters, individual percent transmittance values will be maintained at each of the five specified wavelengths. Furthermore, means and standard deviations will be maintained for each of the percent transmittance values of the linearity filters.

Control standards data may be updated periodically with filter scan data. When new control data exceeds 25 sets of values, then the oldest sets of control data will be removed.

4.4.4 Precision and Accuracy Evaluation

Tristimulus Integrator Performance --

As many as ten replicate measurements may be made on a particular filter. A statistical mean will be computed for each tristimulus value of the examined filter.

Three data will be used in precision evaluation of each tristimulus value. These data are: 1) mean of replicate measurements; 2) historical control mean; and 3) standard deviation from the historical control mean. The absolute value difference between the mean of the replicate measurements and the historical control mean will be evaluated. If this (positive) difference is less than two times the standard deviation, then the tristimulus measurement will be considered acceptable. If the difference is between two and three standard deviations, a warning will be in effect. A difference which exceeds three standard deviations will imply that the measurement is out of control.

Accuracy evaluation will be done in the same way as the precision evaluation. The same control limits will be applied using the following data: 1) mean of the replicate measurements; 2) NBS certified value; and 3) standard deviation from the historical control mean.

Transmittance Measurement Performance --

As many as ten replicate measurements may be made on a given filter. A statistical mean percent transmittance will be determined at each of the five wavelengths specified for the filter. Precision and accuracy evaluations will be made as noted above. Certified NBS values and historical control data for the NBS Linearity Filters will be used in evaluating the measurements.

4.4.5 Report Requirements

Filter Scan Reports --

After each set of filter scans, a temporary report will be available for terminal and/or lineprinter output. This report will include:

- NBS certified values and historical control standards data
- sample filter scan values
- difference between NBS and filter scan values
- difference between control standards and filter scan values
- analyst and date.

Summary Report --

A summary performance evaluation report will be generated at the lineprinter. This report will include all filter report information as well as:

- report date
- analyst
- most recent control standards update date
- control and sample trichromatic coefficients
- precision and accuracy differences
- precision and accuracy determination of acceptable, warning, or out of control, based on the limits described in Section 4.4.4 (Precision and Accuracy Evaluation).

4.5 CHLOROPHYLL ANALYSIS

4.5.1 Existing Method

The methods presently used in chlorophyll analysis include *in vivo* measurement by fluorometry, and acetone extract (*in vitro*) measurements by either fluorometry or spectrophotometry.[5] Such methods facilitate the determination of the relative amount of algal standing crop by observing the amounts of chlorophyll *a*, and for some algae, chlorophylls *b* and *c*.

The trichromatic method is to be automated. This method consists of measuring concentrations of chlorophyll (CHL) extract in aqueous acetone (*in vitro*) by means of spectrophotometry. In particular, any one of three computational methods may be used to compute the concentrations. These methods are the UNESCO,[5] the Strickland-Parsons,[6] and the Jeffrey-Humphrey.[7] Any one of the three methods may be chosen by the analyst.

In the trichromatic method, absorbance is measured at each of three specified wavelengths (see Table 3). The absorbance at 750 nanometers is subtracted from each absorbance reading in order to correct for sample turbidity. These adjusted absorbance values are used in the method trichromatic equations (see Table 4) to compute CHL *a*, *b*, and *c*. To correct for pheophytin *a* -- a degradation product of CHL *a* -- the sample is acidified and measured at approximately 665 nanometers (peak). This value, with the subtraction of absorbance at 750 nanometers for sample, turbidity is used in the appropriate monochromatic equation (see Table 4) to determine the correct concentration of CHL *a*.

4.5.2 Data Acquisition and Reduction

Automated Method --

Before analysis of chlorophyll begins, wavelength calibration may be checked and reset if necessary. Transmittance calibration may also be checked and reset. The system must ensure the establishment of certain parameters, which include: sample ID, sample type (plankton or periphyton), computational method to be used, dilution factor, extract volume, cell path length, sample volume or area, and biomass (optional).

TABLE 3. WAVELENGTHS USED IN THE TRICHROMATIC COMPUTATIONAL METHODS

Method	CHL *a* (peak)	CHL *b*	CHL *c*	PHEO *a* (peak)
UNESCO	663	645	630	665X*
Strickland-Parsons	665	645	630	665X
Jeffrey-Humphrey	664	647	630	665X

*X refers to the fact that this reading is taken after acidification.

Scans on samples will be made using automated scanning. Transmittance values will be collected over the necessary wavelength range required by the computational methods. These data will be reduced to absorbance values at integer nanometer increments. The relationship between transmittance and absorbance is such that:

$$A = LOG_{10}(1/T)$$

where A is absorbance and T is transmittance. (Percent transmittance is 100T.)

Manual Entry Alternative Method --

A manual data-entry alternative will be provided. The same parameters will be required by the system as in the automated method. Absorbance or

TABLE 4. EQUATIONS USED IN THE TRICHROMATIC COMPUTATIONAL METHODS

Method		Equations
UNESCO	CHL a	$= F^{\#}x(11.64A^{*}_{663} - 2.16A_{645} + 0.10A_{630})$
	CHL b	$= Fx(-3.94A_{663} + 20.97A_{645} - 3.66A_{630})$
	CHL c	$= Fx(-5.53A_{663} - 14.81A_{645} + 54.22A_{630})$
	PHEO a	$= Fx(26.73\ (1.7A_{665X} - A_{663}))$
	CHL a'**	$= Fx(26.73\ (A_{663} - A_{665X}))$
Strickland-Parsons	CHL a	$= Fx(11.6\ A_{665} - 1.31A_{645} - 0.14A_{630})$
	CHL b	$= Fx(-4.34A_{665} + 20.7A_{645} - 4.42A_{630})$
	CHL c	$= Fx(-4.64A_{665} - 16.30A_{645} + 55A_{630})$
	PHEO a	$= Fx(26.73\ (1.7A_{665X} - A_{665}))$
	CHL a'	$= Fx(26.73\ (A_{665} - A_{665X}))$
Jeffrey-Humphrey	CHL a	$= Fx(11.85A_{664} - 1.54A_{647} - 0.08A_{630})$
	CHL b	$= Fx(-5.43A_{664} + 21.03A_{647} - 2.66A_{630})$
	CHL c	$= Fx(-1.67A_{664} - 7.6A_{647} + 24.52A_{630})$
	PHEO a	$= Fx(26.73\ (1.7A_{665X} - A_{664}))$
	CHL a'	$= Fx(26.73\ (A_{664} - A_{665X}))$

#F is an analysis constant such that: F = (E x D)/(L x S x 1000), where E is extract volume (ml), D is the dilution factor, L is the cell path length (cm), and S is sample volume (cubic meters) for plankton or sample area (square meters) for periphyton.

* A_{NNN} denotes absorbance at wavelength of NNN nanometers.

** CHL a' designates CHL a concentration corrected for PHEO a.

transmittance values will be provided by the analyst at the wavelengths specified by the selected method of computation.

4.5.3 Peak Absorbance Choice

The three available methods of computation differ slightly with respect to the expected peak absorbance wavelength, that is, the wavelength at which absorbance is highest. The analyst will be permitted to use either the method peak absorbance at the specified wavelength, or the measured peak absorbance. This option will apply to both the automated and manual entry methods. The decision will apply to all samples of a chlorophyll run.

4.5.4 Replicate Statistics

Precision may be established through repeated scans of a given sample. Up to five scans (replicates) may be made. Replicate statistics for each sample are to be computed and maintained. These statistics include sample mean, standard deviation, and percent relative standard deviation. The statistics are to be computed for amounts of chlorophylls a, b, c, and total chlorophyll, and for ratios of chlorophylls a to b, and a to c. Statistics will also be calculated for the amount of pheophytin a, and the ratio of peak absorbances before and after acidification. If biomass is a factor, statistics will be maintained for biomass and autotrophic index. An option will be provided to delete or add a sample replicate and recalculate statistics.

4.5.5 Report and Plot Requirement

Sample and Summary Reports --

Optionally accessible reports will be prepared for each sample and for all samples of a run. Each type of report will provide the following headings: analyst; analysis date; station/study; sample ID; sample type; extraction method and extract volume (milliliters); computational method; cell path length (cm.); and instrument. Both types will be available on terminal and/or lineprinter.

Sample reports will add the heading of dilution factor. Listed results from scans before acidification (correction) will include replicate values, means, and standard deviations for raw data absorbances and replicate volume/area, chlorophylls a, b, c, and total chlorophyll milligrams per cubed or squared meter), and ratios of chlorophylls a to b, and a to c. Results of post-acidified replicates will include replicate volume/area, pheophytin a, chlorophyll a, total chlorophyll, and ratios of chlorophylls a to b and a to c. If biomass is an input, it will be listed (grams per cubed or squared meter) along with the autotrophic index.

Summary reports will list mean values for all samples. The data per sample will include: sample ID; chlorophyll a (uncorrected and corrected); chlorophylls b and c; pheophytin a; and total chlorophyll.

Sample Plot --

A plot may be prepared for any replicate of a sample. This plot may be a simple response curve listing or may be a more formal linear plot with wavelength on the horizontal axis and absorbance on the vertical axis. The following information will be included with the plot: sample ID, analyst, analysis date, sample type (plankton or periphyton), and replicate number. If the sample is acidified, both unacidified and acidified data will be plotted on the same graph. All other information will be supplied in the summary report.

4.5.6 Sample File Control Interaction

The EPA Sample File Control (SFC) system obtains analysis results from various USEPA laboratories and transfers the information to a data base. In order to be compatible with SFC, the UVVIS system must create run results files which contain certain information organized in a defined manner.[8]

In the event that SFC interaction is required, a sample ID will be retained for each sample that is run. The eight character sample ID will be provided by the analyst (or generated by the system) for each sample, and will be stored in the appropriate run results file. Due to the uncertainty of the transfer of biological data to a data base, the module for SFC interaction was designed into the UVVIS automation system. However, the final detailed implementation of the module will be accommodated at a future date.

4.6 COLOR ANALYSIS

It is desirable to measure the aesthetic quality of environmental samples -- particularly that of industrial wastes. Also, by Federal law, Section 304g, PL 92-500, the USEPA has been required to develop a procedure for measuring the color of water.

4.6.1 Existing Method

The Colorometric Platinum-Cobalt method,[9] established by the American Public Health Association (APHA), has been adequate for the evaluation of yellow color levels in river and stream waters. This method entails the comparison of samples to standard APHA references (colored with set amounts of a platinum-cobalt solution). The APHA method, however, may be quite inexact when industrial samples are analyzed.

The American Dye Manufacturers Institute (ADMI) developed an alternative method for measuring color levels in water.[10] The ADMI method was designed to meet the following four criteria:

1. The method should be applicable to any color (hue);

2. The method should be sensitive to small color differences;

3. The method results should be related to APHA colorometric units (platinum-colbalt standards); and

4. The method should require relatively inexpensive instrumentation.

The USEPA adopted this method in fulfillment of the Federal law mentioned above.

The ADMI method of color evaluation consists of five basic operations. (Terminology and equations in the method are explained in the noted reference materials.) The method steps are:

1. Measurement of the visible spectrum of the sample on a suitable instrument;

2. Calculation of CIE tristimulus values,[11] denoted as X, Y, and Z;

3. Renotation of tristimulus values to Munsell coordinates,[12] Vx, Vy, and Vz;

4. Using Munsell coordinates, calculation of the Adams-Nickerson[13] color difference number DE; and

5. Conversion of DE to ADMI values using standards curve -- the curve relating ADMI values (of APHA standards) to corresponding DE numbers.

4.6.2 Data Acquisition and Reduction

Automated Method --

Before analysis of sample unknowns begins, wavelength calibration may be checked or set, and transmittance calibration may be performed. Following these procedures, update standards, check standards, and unknowns may be run.

The five steps of the ADMI method are to be followed in the automated acquisition and reduction of color data. These steps are:

1. Measurement of the visible spectrum of the sample on a scanning spectrophotometer;

2. Conversion of transmittance data to tristimulus values X, Y, and Z by means of the 30 Selected Ordinate[11] or Weighted Ordinate[10] spectrophotometric method;

3. Conversion of tristimulus values X, Y, and Z to Munsell coordinates Vx, Vy, and Vz;

4. Calculation of Adams-Nickerson color difference number (DE) by the formula;[13]

$$DE = \left(\left[0.23 \times \Delta Vy \right]^2 + \left[\Delta (Vx - Vy) \right]^2 + \left[0.4 \times \Delta (Vy - Vz) \right]^2 \right)^{1/2} ;$$

5. Conversion of DE to ADMI value by the standards curve (ADMI = f(DE)) established through calibration.

Manual Entry Alternative Method--

The Three Filter Tristimulus Method[9] may be used in lieu of steps 1 and 2 of the automated method (described above). This method uses an instrument which measures sample color with a source beam passed through each of three different filters. The resulting values are used in equations to calculate pseudo-tristimulus values X, Y, and Z. The remaining steps (3 through 5) of the automated method are then followed.

4.6.3 Standards and Quality Control

Control standards for six ADMI values -- 25, 50, 100, 150, 200, and 250 -- will be maintained. As many as six replicates per standard may be stored, and statistics on the standard replicates will be maintained.

Check standards may be run. If the mean DE value of the check standard is below plus/minus two times the standard deviation of the control standards mean DE, then the check standard will be considered acceptable, that is, in control. If the check standard mean DE is between plus/minus two and three standard deviations, a warning should be issued. The check standard will be considered out of control when the mean DE is beyond plus/minus three standard deviations.

4.6.4 Report and Plot Requirements

Intermediate Standards Reports --

Brief terminal reports will accompany each update and check standard. The standard ADMI value, mean standard DE number and its standard deviation, measured DE number and its computed ADMI value, and the control rating will be displayed for each update or check standard.

For a set of update standards, a terminal or lineprinter report will be available. For each APHA standard, this report will list: mean DE number and its standard deviation; mesured DE number of the update standard and its difference from the mean; and the control rating.

For a set of check standards, a terminal or lineprinter report may be acquired. For each check standard, the report will include: ADMI value of the APHA standard, expected and measured DE numbers and their difference, standard deviation of the mean (expected) DE, and the control rating.

Intermediate Unknowns Report --

For each unknown, a terminal display will list sample I.D., measured DE number, and calculated ADMI value. For any set of unknowns, a more

comprehensive terminal/lineprinter report will be made available. For each unknown, this report will display: sample I.D., measured DE, calculated ADMI, and any accompanying comments.

Summary Standards Report --

Existing standards data will be accessible through a summary standards report, whether or not update or check standards are run. The report will display the calibration equation (ADMI=f(DE)), the standard error of estimate, and the percent relative standard error of estimate. In addition, the DE numbers of all replicates will be listed for each APHA standard, along with their mean DE numbers and standard deviations. Dates for each replicate set of standards will also be noted.

General Summary Report --

A summary report will be available. It will list the following information: standards calibration equation and standard error of estimate, new standards statistics, and data of check standards and unknowns as in the intermediate reports.

Plots --

Information will be maintained for each new standard, check standard, and unknown for plot generation. These plots will be formal linear plots with transmittance on the vertical axis, and wavelength on the horizontal axis. (For non-plotting terminals, a listed response curve should be available.) Information to be supplied with the plot will include: analysis date, analyst, type of plot (update standard, check standard, or unknown); measured DE and ADMI values, and APHA standard ADMI for update and check standards.

4.7 SAMPLE TRANSMITTANCE/ABSORBANCE ANALYSIS

Any sample may be analyzed for transmittance or absorbance. The data may be obtained over any specified wavelength range within the visible light range (that is, between 380 and 800 nanometers).

4.7.1. Data Acquisition and Reduction

Before analysis begins, wavelength calibration may be checked. Transmittance at 0% and 100% transmittance levels may be calibrated.

The wavelength range, specified by the analyst, may be at the minimum 50 nanometers, and at the maximum 420 nanometers (over the region of 380 to 800 nanometers). These parameters must be established prior to the transmittance calibration since the calibration must encompass the range.

Transmittance values will be acquired through automated scanning. These data will be reduced to percent transmittance values at integer nanometer increments. Conversion of percent transmittance to absorbance will then be made if necessary.

Report and Plot Requirements

Sample Report --

The analyst may request a listing of transmittance or absorbance values as a function of wavelength. The report may be received at terminal and/or line printer and will include the additional information of sample I.D., sample comment, analyst, and analysis date.

Summary Report --

A summary line printer report may be obtained. This report will contain the following information:

- name of analyst
- sample ID for each sample
- sample analysis date and report date
- sample comment for each sample
- whether transmittance or absorbance was computed (for each sample)

Sample Plots --

A formal linear plot may be obtained for each sample. The plot will display transmittance or absorbance on the vertical axis and wavelength on the horizontal axis. Additional information will include: sample ID, analysis date, analyst, and sample comment. A response curve listing may also be made.

SECTION 5

FUNCTIONAL DESIGN

5.1 GENERAL DESCRIPTION OF THE UVVIS SYSTEM

The Ultraviolet-Visible Spectrophotometer (UVVIS) automation system is constructed modularly. It is composed of basic function modules which serve to control the UVVIS system, perform major types of analysis, and gracefully exit the system. The Master Controller is the base module of the system through which the user gains access to all other function modules. Figure 1 exhibits the general scheme of the UVVIS system.

Figure 1. General scheme of the UVVIS system.

5.1.1 Master Control Level

The operation of the UVVIS system begins at master control level. (The Master Control module is in charge at this level.) The user is presented with a set of seven options and makes a selection. The options initiate the following functions: 1) analyze spectrophotomer quality assurance; 2) analyze chlorophyll with automated spectrophotometer; 3) analyze color with automated spectrophotometer; 4) analyze chlorophyll by manual data-entry; 5) analyze color by manual data-entry; 6) plot chlorophyll or color scans; and 7) end the UVVIS session.

After the completion of any of the functions, except for the seventh option, the Master Controller resumes power. The set of options is again presented to the user. For any automated selection (that is, 1-5 above), an initial check of the automated spectrophotometer is possible.

5.1.2 Set-Up and Instrument Checkout

The-set up and checkout procedure is in three optional parts. The first part is the presentation of elementary set up instructions, concerned with ensuring that all manual switches are in the correct positions.

The second portion of the procedure entails testing signals received from the channels, and verifying operation of the instrument's scanning mechanism. A/D signals from the transmittance and wavelength signal channels should be in the range of 500 to 15500 A/D counts. And a four-second scan should yield a change of 10 or more A/D counts. An automated run may not begin until the computer verifies that the spectrophotometer and its interface unit are working properly.

The third part consists of the wavelength calibration and checking procedures. These procedures are described in section 5.3.1, Wavelength Calibration.

5.2 GENERAL DESIGN OF THE ANALYSIS MODULES

The three analysis functions-- quality assurance, chlorophyll, and color-- are all of a similar design. (See Figure 2.) Each function begins with the initialization process, which entails gathering certain information, allocating data storage space, and in some cases setting certain indicator flags. After the initialization, one may analyze a sample, check and/or set instrument calibration parameters, receive a summary report, or end the analysis and return to the master control level. Samples may be filters, standards, or unknowns, depending on the type of analysis.

Included among the options of any automated function is the ability to check and/or establish transmittance calibration. Also, each automated analysis has an optional routine to check and/or set the scan speed.

It should be noted that the same wavelength calibration data will be used in every specific automated function. However, scan speed and transmittance calibration will be tailored to fit the needs of each type of automated analysis. This feature enables one to set specific scan speeds for different procedures, depending on the degree of precision desired. Also, different analyses may require various types of sample references, for example, air for quality assurance, distilled water for color, and reagent for chlorophyll. In this way, transmittance calibration data will be based on a particular type of sample reference.

It should also be noted that an additional option is available in automated and manual chlorophyll analysis. One may choose to delete data pertaining to a particular sample.

The same data reduction technique is used in all the analysis modules. Basically, the transmittance signals for the 0% and 100% levels at integer nanometer increments are stored. Sample scan data are reduced to this form. By interpolation, the percent transmittance of the sample at integer nanometers can be computed. The percent transmittance values are then subjected to a least squares regression smoothing technique[14] in order to remove "signal noise" from the data. (The 5-point smooth will be used.)

5.3 DESIGN OF CALIBRATION SETTING AND CHECKING PROCEDURES

5.3.1 Wavelength Calibration

To calibrate a spectophotometer for wavelength, a correspondence between wavelength values (in nanometers) and A/D counts must be established. This will be done by means of least squares linear regression. The process contains several steps.

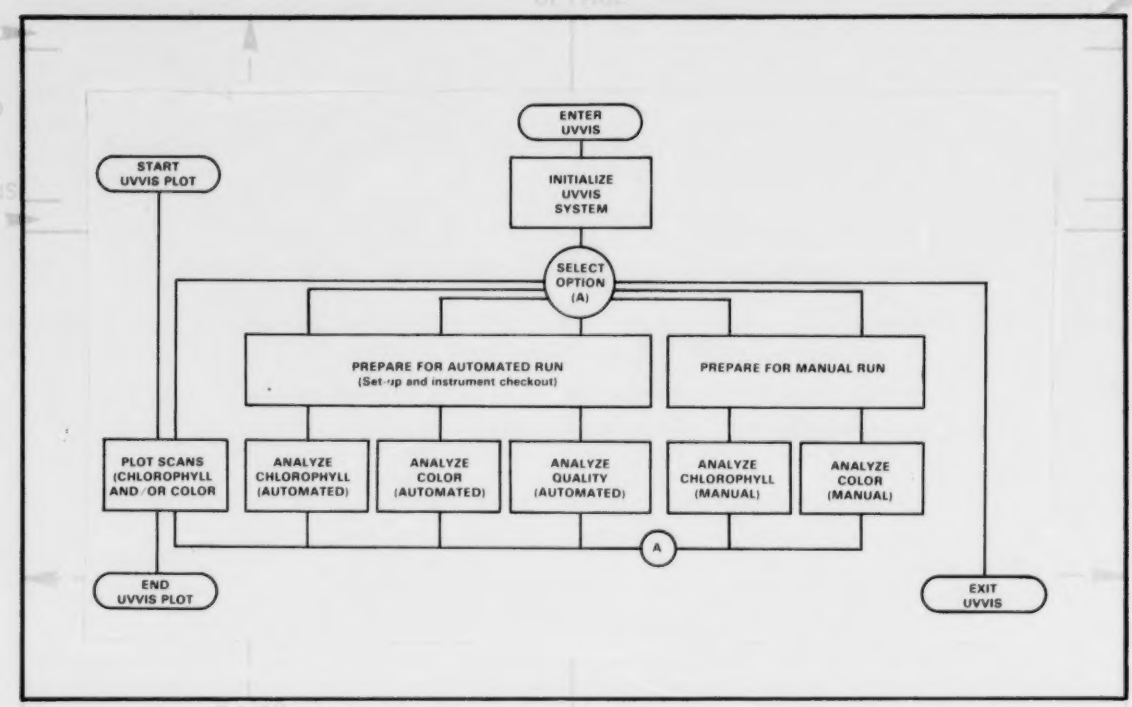


Figure 1. General scheme of the UVVIS system.

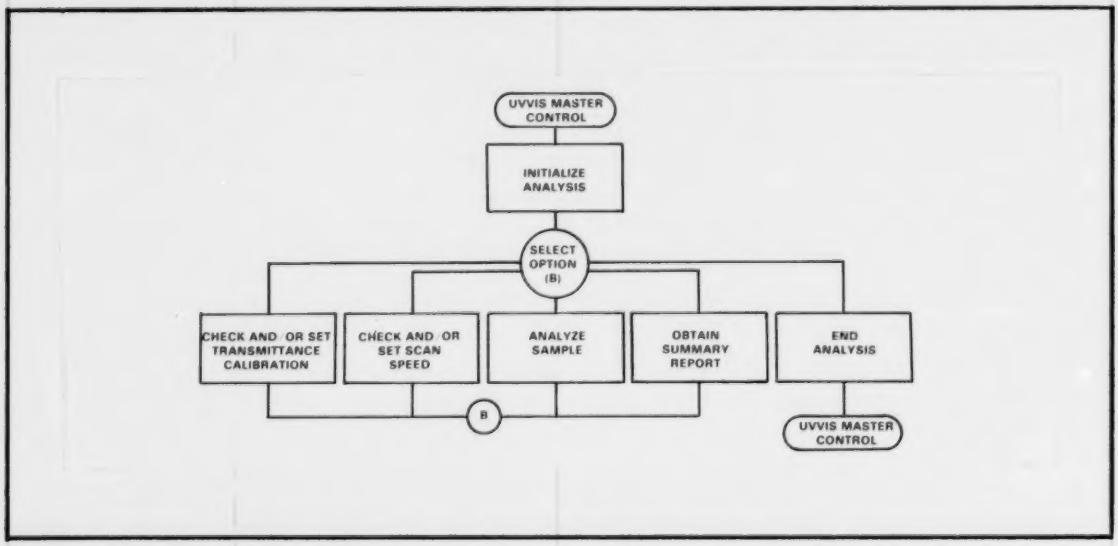


Figure 2. General design of the UVVIS analysis modules.

First, the analyst chooses to take readings at 50-nanometer or 100-nanometer increments. (The wavelength range is already set at 300 to 800 nanometers.) The analyst also decides if one, two, or three readings are to be taken at each point.

Second, the wavelength dial is set and the A/D count measured (by the computer) for each setting. This process is repeated if more than one reading per point is to be taken.

Third, the coefficients of the second-degree equation are computed, along with the standard error of estimate. (The standard error of estimate helps determine normal human inaccuracy in setting the wavelength.)

Finally, the data are stored, dated, and the calibration results are displayed. (Stored data include the three quadratic coeffieients and the standard error of estimate in nanometers, as well as the date and time of calibration.)

Checking Calibration --

Checking of the wavelength calibration is done one point at a time, and may be repeated indefinitely. The analyst sets the wavelength, and enters the nanometer value into the computer. The automated system measures the A/D count, and calculates the measured wavelength value according to the wavelength calibration equation. The absolute value of the difference between the expected and measured wavelength values is computed. If the absolute value difference is 0.25 nm. or less, the wavelength calibration is in control. If the absolute value difference is between 0.25 nm. and 0.5 nm., a warning is given to the operator. If the absolute value difference is greater than 0.5 nm., the wavelength calibration is noted out of control.

5.3.2 Scan Speed

Setting the scan speed is a short operation. A ten-second scan is made. Wavelengths after two, six, and ten seconds of scanning are determined. Then scan speeds (in nanometers-per-minute) for the 2-6 second and 6-10 second scans are determined. If the two speeds differ by no more than 0.5%, then an integer to the nearest five nanometers-per-minute will be stored, dated, and displayed.

Checking Scan Speed --

Checking the scan speed is repeatable. A five-second scan is made, and the speed computed from measured wavelengths after one and five seconds. The expected and computed scan speeds along with the percent difference from the expected speed are displayed.

5.3.3. Transmittance Calibration

For each type of analysis, transmittance may be calibrated to correct for the effects of various factors on instrument measurement. Several steps are entailed in this calibration procedure.

First, the wavelength range must be determined. The upper and lower bounds are established before the sample analysis begins. Table 5 lists the wavelength bounds.

TABLE 5. TRANSMITTANCE CALIBRATION WAVELENGTH RANGES

Type of Analysis	Wavelength Range (in nanometers)
Quality Assurance	400-700
Chlorophyll	620-760
Color	400-700

Second, the analyst sets the wavelength at the computer-displayed midpoint of the scan range, places a blockade in front of the light source beam, and adjusts the meter to 0% transmittance. Then the blockade is removed, and the meter adjusted to 100% transmittance. (For both settings, a cuvette of reagent or distilled water is placed in the sample reference chamber, and in the sample chamber at 100% transmittance, if the particular type of analysis so requires.)

Third, the wavelength is set to a computer-displayed position which is just above the upper bound of the scan range, and the scan is initiated. Then, the wavelength is reset to the starting position, the beam blockade again inserted, and a second scan initiated. Data are collected, reduced, and stored upon user request.

Checking Calibration --

Checking may be done before or during an analysis session. To begin, the second step of the calibration procedure (described above) is done. Any number of readings may then be taken. The analyst sets the wavelength at any position within the scan range, and places a blockade or appropriate blank in the sample chamber (for 0% or 100% transmittance). The computer calculates the percent transmittance and displays it at the terminal.

5.4 OPTIONAL QUALITY ASSURANCE FILTER TEST

5.4.1. Description of an Automated Run

This analysis is used to evaluate the quality of an automated spectrophotometer's measurements through tests of its precision and accuracy. Following optional wavelength calibration and checking procedures (explained in "General Design of the Analysis Modules"), the tests may begin.

The analyst selects one of the appropriate NBS Tristimulus or Linearity filters, and enters its number into the computer. A scan is made throughout the range of 400 to 700 nanometers, and transmittance data are reduced and stored. For the NBS Tristimulus filters, tristimulus values X, Y, and Z are computed. For the NBS Linearity filters, percent transmittance at each specified wavelength is determined.

A brief report is sent to the terminal, giving statistics on precision and accuracy, based on sample data, historical control data, and NBS values. The sample data may then be stored in the file of historical control data (at which point new control statistics are computed). When the last sample has been analyzed, a summary report may be received at the terminal and/or lineprinter.

5.4.2 Example Reports

Figures 3 and 4 exhibit intermediate report formats for an NBS Tristimulus filter and an NBS Linearity filter, respectively. A summary report format is shown in Figure 5.

5.4.3 Data Files

Two data files are needed to store NBS filter data. One file contains NBS certified values for the tristimulus filters and for the linearity filters. This file is required for accuracy evaluations. The other file maintains up to twenty-five sets of historical data for each type of filter. For each set, mean values -- tristimulus or percent transmittance -- are maintained, along with standard deviations on each mean. This file contains information needed for rating precision.

A data summary file is also kept. This file is used to prepare the summary report.

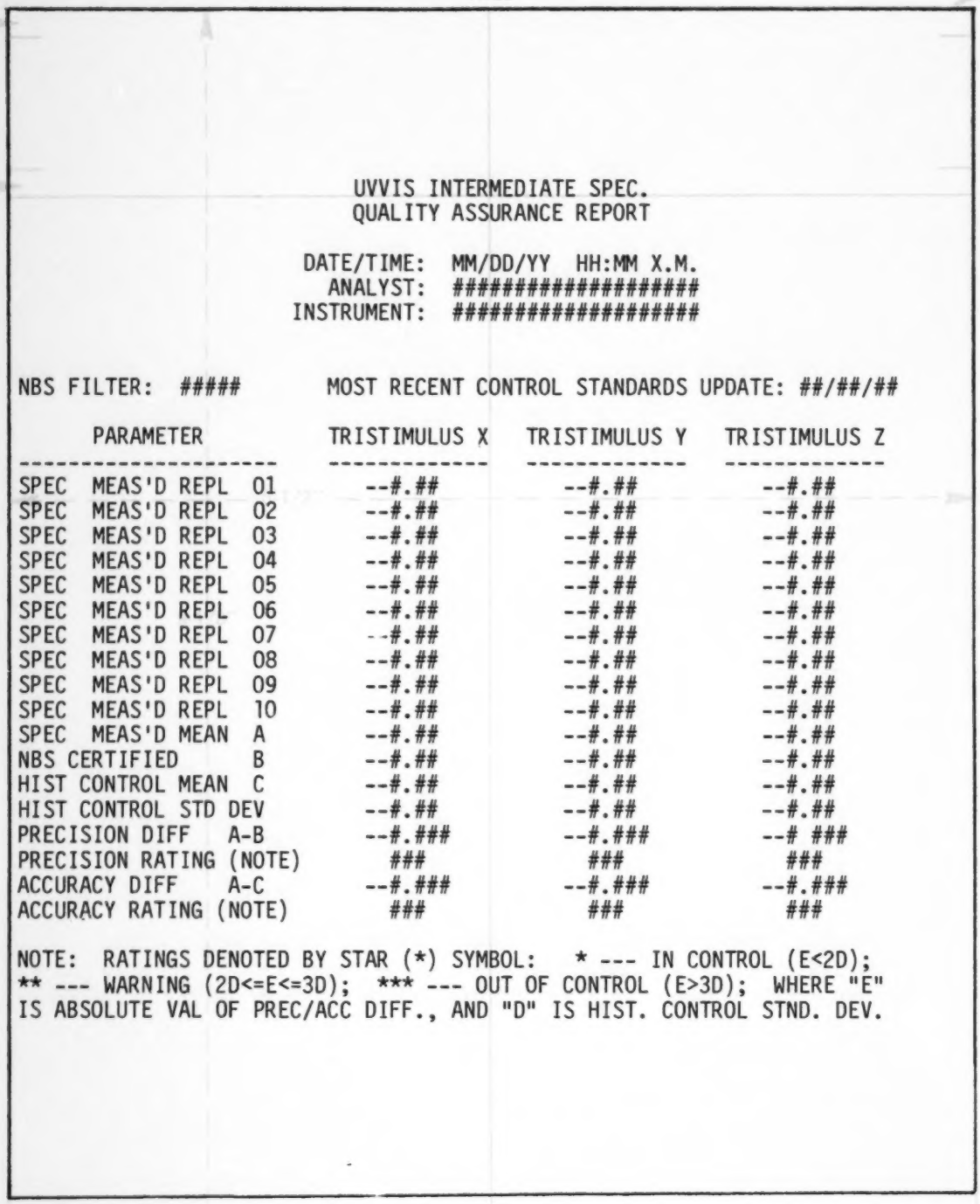
5.5 OPTIONAL CHLOROPHYLL ANALYSIS

5.5.1 Description of an Automated Run

Normal calibration checking and setting procedures may preceed sample analysis. For a run of several samples, certain parameters may be examined and changed if necessary. These parameters are: station or study; sample type (plankton or periphyton), extraction method, extract volume (liters), cell path length (cm.), computational method, comment, whether or not the samples are to be acidified, whether method or found peak absorbance is desired, and whether or not biomass is to be determined. Once established, the values become defaults for future runs, but can always be altered. The analysis is controlled through various commands. (See Figure 6.)

For each sample, only the sample ID and dilution factor need be established. (The system can create a sample ID automatically.) The only other possible variable factors apply to individual replicates. These factors may be sample volume or area (liters or squared meters) and, if measured, biomass (milligrams per liter or grams per squared meter). Data from a sample may be deleted by command. A report summarizing information on all samples is available by command.

For each sample, a maximum of five valid replicates will be examined. Each examination will entail the reduction of data acquired from a scan of 760 to 620 nanometers. (For acidified samples, a second scan is made after treatment of the replicate.) Replicate data may be annulled after each



UVVIS INTERMEDIATE SPEC.
QUALITY ASSURANCE REPORT

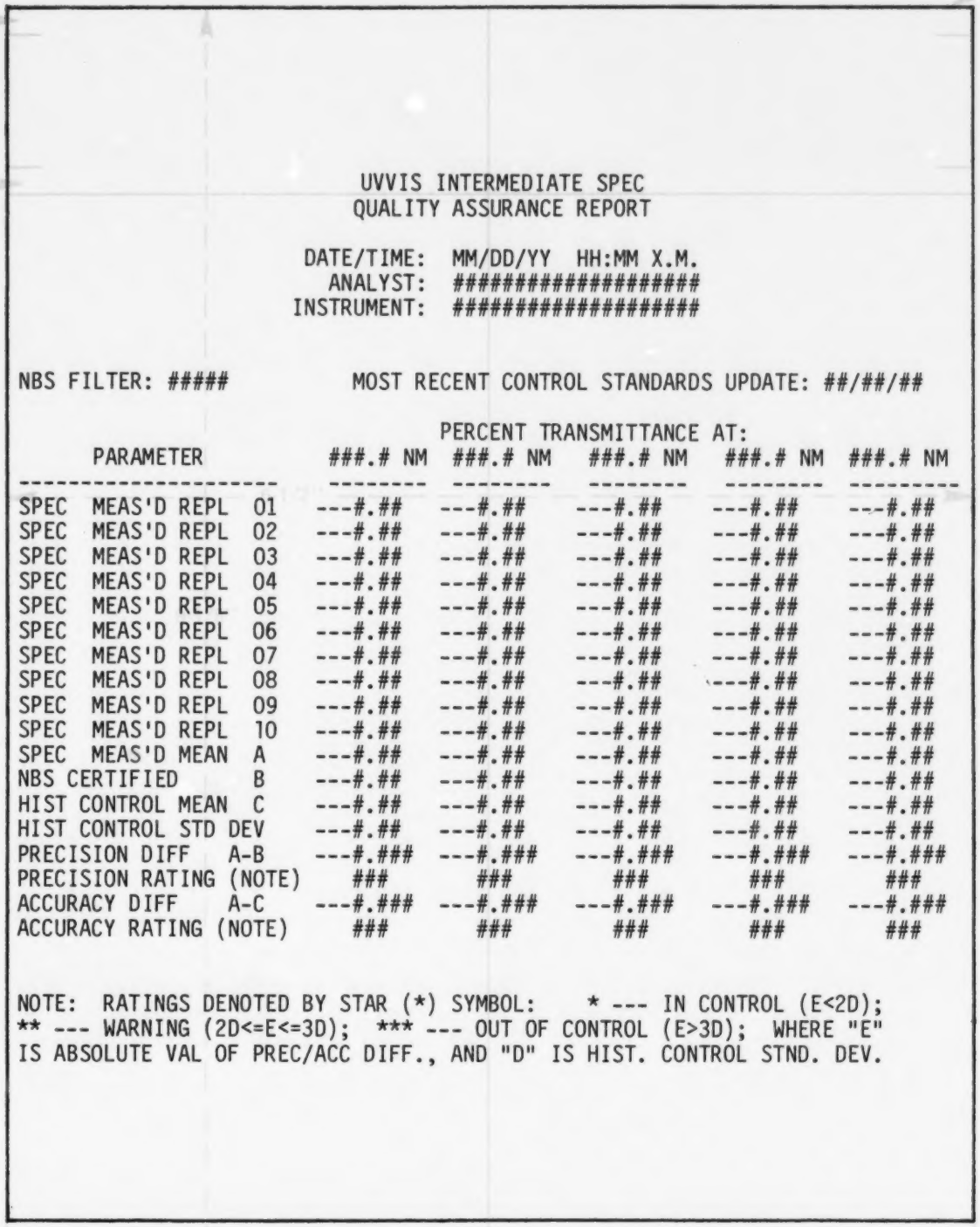
DATE/TIME: MM/DD/YY HH:MM X.M.
ANALYST: ##################
INSTRUMENT: ##################

NBS FILTER: ##### MOST RECENT CONTROL STANDARDS UPDATE: ##/##/##

PARAMETER		TRISTIMULUS X	TRISTIMULUS Y	TRISTIMULUS Z
SPEC MEAS'D REPL	01	--#.##	--#.##	--#.##
SPEC MEAS'D REPL	02	--#.##	--#.##	--#.##
SPEC MEAS'D REPL	03	--#.##	--#.##	--#.##
SPEC MEAS'D REPL	04	--#.##	--#.##	--#.##
SPEC MEAS'D REPL	05	--#.##	--#.##	--#.##
SPEC MEAS'D REPL	06	--#.##	--#.##	--#.##
SPEC MEAS'D REPL	07	--#.##	--#.##	--#.##
SPEC MEAS'D REPL	08	--#.##	--#.##	--#.##
SPEC MEAS'D REPL	09	--#.##	--#.##	--#.##
SPEC MEAS'D REPL	10	--#.##	--#.##	--#.##
SPEC MEAS'D MEAN	A	--#.##	--#.##	--#.##
NBS CERTIFIED	B	--#.##	--#.##	--#.##
HIST CONTROL MEAN	C	--#.##	--#.##	--#.##
HIST CONTROL STD DEV		--#.##	--#.##	--#.##
PRECISION DIFF	A-B	--#.###	--#.###	--# ###
PRECISION RATING (NOTE)		###	###	###
ACCURACY DIFF	A-C	--#.###	--#.###	--#.###
ACCURACY RATING (NOTE)		###	###	###

NOTE: RATINGS DENOTED BY STAR (*) SYMBOL: * --- IN CONTROL (E<2D); ** --- WARNING (2D<=E<=3D); *** --- OUT OF CONTROL (E>3D); WHERE "E" IS ABSOLUTE VAL OF PREC/ACC DIFF., AND "D" IS HIST. CONTROL STND. DEV.

Figure 3. Intermediate Quality Assurance report format for an NBS tristimulus filter.



UVVIS INTERMEDIATE SPEC
QUALITY ASSURANCE REPORT

DATE/TIME: MM/DD/YY HH:MM X.M.
ANALYST: ##################
INSTRUMENT: ##################

NBS FILTER: ##### MOST RECENT CONTROL STANDARDS UPDATE: ##/##/##

PARAMETER			PERCENT TRANSMITTANCE AT: ###.# NM	###.# NM	###.# NM	###.# NM	###.# NM
SPEC	MEAS'D REPL	01	---#.##	---#.##	---#.##	---#.##	---#.##
SPEC	MEAS'D REPL	02	---#.##	---#.##	---#.##	---#.##	---#.##
SPEC	MEAS'D REPL	03	---#.##	---#.##	---#.##	---#.##	---#.##
SPEC	MEAS'D REPL	04	---#.##	---#.##	---#.##	---#.##	---#.##
SPEC	MEAS'D REPL	05	---#.##	---#.##	---#.##	---#.##	---#.##
SPEC	MEAS'D REPL	06	---#.##	---#.##	---#.##	---#.##	---#.##
SPEC	MEAS'D REPL	07	---#.##	---#.##	---#.##	---#.##	---#.##
SPEC	MEAS'D REPL	08	---#.##	---#.##	---#.##	---#.##	---#.##
SPEC	MEAS'D REPL	09	---#.##	---#.##	---#.##	---#.##	---#.##
SPEC	MEAS'D REPL	10	---#.##	---#.##	---#.##	---#.##	---#.##
SPEC	MEAS'D MEAN	A	---#.##	---#.##	---#.##	---#.##	---#.##
NBS CERTIFIED		B	---#.##	---#.##	---#.##	---#.##	---#.##
HIST CONTROL MEAN		C	---#.##	---#.##	---#.##	---#.##	---#.##
HIST CONTROL STD DEV			---#.##	---#.##	---#.##	---#.##	---#.##
PRECISION DIFF		A-B	---#.###	---#.###	---#.###	---#.###	---#.###
PRECISION RATING (NOTE)			###	###	###	###	###
ACCURACY DIFF		A-C	---#.###	---#.###	---#.###	---#.###	---#.###
ACCURACY RATING (NOTE)			###	###	###	###	###

NOTE: RATINGS DENOTED BY STAR (*) SYMBOL: * --- IN CONTROL (E<2D); ** --- WARNING (2D<=E<=3D); *** --- OUT OF CONTROL (E>3D); WHERE "E" IS ABSOLUTE VAL OF PREC/ACC DIFF., AND "D" IS HIST. CONTROL STND. DEV.

Figure 4. Intermediate Quality Assurance report format for an NBS linearity filter.

UVVIS

SPECTROPHOTOMETER QUALITY ASSURANCE SUMMARY

DATE/TIME: MM/DD/YY HH:MM X.M.
ANALYST: ##########
INSTRUMENT: ##########

NBS FILTER: ##### MOST RECENT CONTROL STANDARDS UPDATE: ##/##/##

PARAMETER	PERCENT TRANSMITTANCE AT: ###.# NM	###.# NM	###.# NM	###.# NM	###.# NM
SPEC MEAS'D MEAN A	---#.##	---#.##	---#.##	---#.##	---#.##
NBS CERTIFIED B	---#.##	---#.##	---#.##	---#.##	---#.##
HIST CONTROL MEAN C	---#.##	---#.##	---#.##	---#.##	---#.##
HIST CONTROL STD DEV	---#.##	---#.##	---#.##	---#.##	---#.##
PRECISION DIFF A-B	---#.###	---#.###	---#.###	---#.###	---#.###
PRECISION RATING (NOTE)	###	###	###	###	###
ACCURACY DIFF A-C	---#.###	---#.###	---#.###	---#.###	---#.###
ACCURACY RATING (NOTE)	###	###	###	###	###

NBS FILTER: ##### MOST RECENT CONTROL STANDARDS UPDATE: ##/##/##

PARAMETER	TRISTIMULUS X	TRISTIMULUS Y	TRISTIMULUS Z
SPEC MEAS'D MEAN A	--#.##	--#.##	--#.##
NBS CERTIFIED B	--#.##	--#.##	--#.##
HIST CONTROL MEAN C	--#.##	--#.##	--#.##
HIST CONTROL STD DEV	--#.##	--#.##	--#.##
PRECISION DIFF A-B	--#.###	--#.###	--#.###
PRECISION RATING (NOTE)	###	###	###
ACCURACY DIFF A-C	--#.###	--#.###	--#.###
ACCURACY RATING (NOTE)	###	###	###

NOTE: RATINGS DENOTED BY STAR (*) SYMBOL: * -- IN CONTROL (E< 2D);
** --- WARNING (2D<=E<=3D); *** --- OUT OF CONTROL (E>3D); WHERE "E"
IS ABSOLUTE VAL OF PREC/ACC DIFF., AND "D" IS HIST. CONTROL STND. DEV.

Figure 5. Summary report format for Quality Assurance analysis.

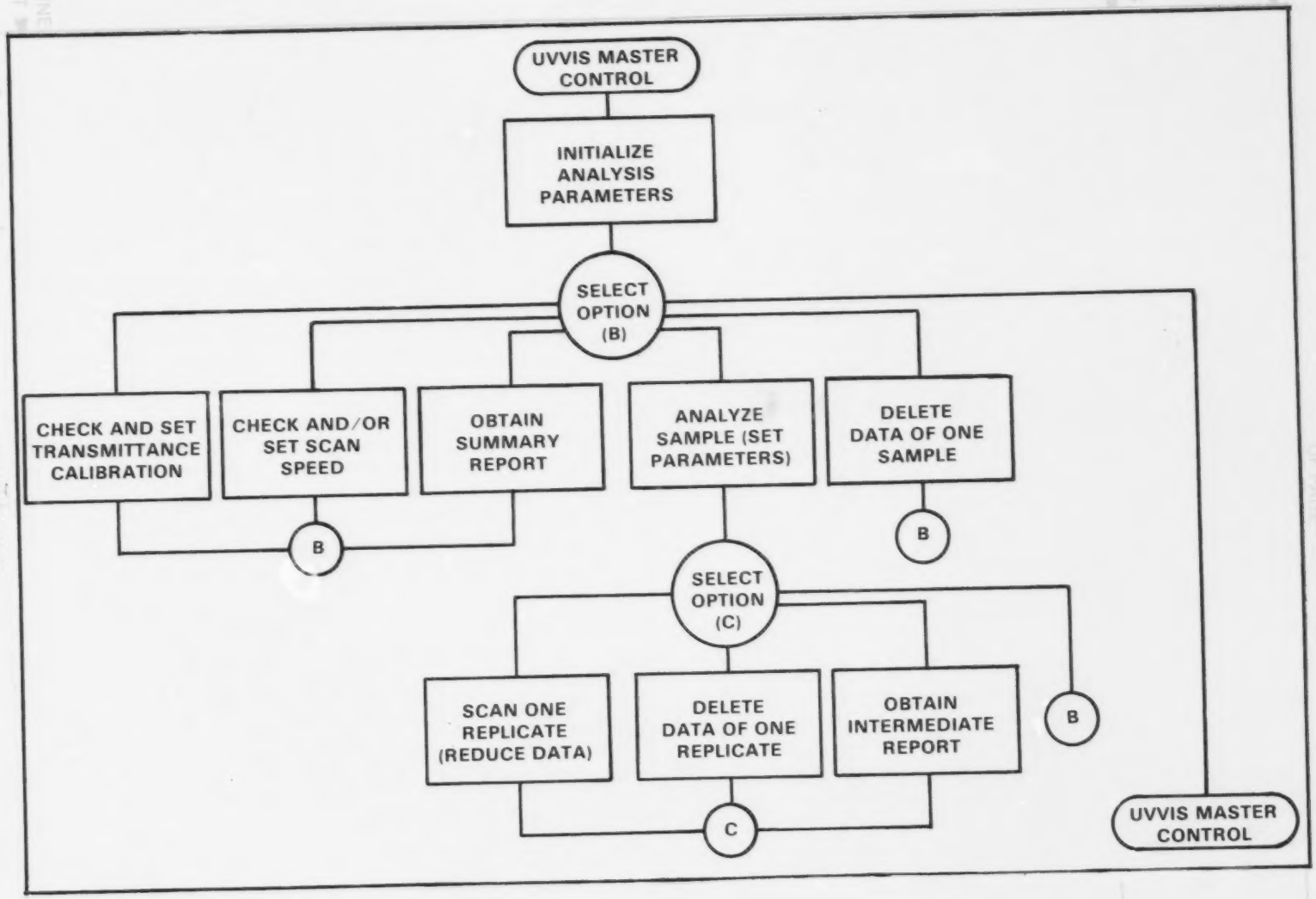


Figure 6. Automated chlorophyll analysis command structure.

evaluation, or later by command. Intermediate reports contain results for all replicates of the particular sample being studied, and are available by command.

5.5.2 Description of a Manual Run

The same parameters are required for a manual analysis as for an automated analysis. The analyst also decides to enter absorbance or transmittance (not percent transmittance) values.

For each sample replicate, values are entered for the three wavelengths of the computational method's trichromatic equations, and for 750 nanometers to correct for sample turbidity. If a sample is acidified, values are required at the wavelength of the appropriate monochromatic equation, and at 750 nanometers. Intermediate and summary reports may be acquired. Replicate and sample data may be deleted by command.

5.5.3 Example Reports and Plots

Figures 7 and 9 show formats of intermediate and summary reports, which indicate all replicates and all samples, respectively. Figure 8 exhibits an example plot which graphically represents data from scans of a replicate before and after acidification.

5.5.4 Data Files

All reduced and evaluated scan data are stored in a Data Summary file. This file will be used to prepare the output reports. In automated runs, plot data and plot reference files are produced. (See section 5.7.)

5.6 OPTIONAL COLOR ANALYSIS

5.6.1 Description of an Automated Run

The automated module has several features (depicted in Figure 10). Historical standards are maintained and can be updated each analysis session. Check standards and unknowns may be run also. Intermediate reports, plots, and a summary report are available each session.

The analyst may add one or more new sets of APHA standards to the historical control standards data file. Each set consists of six different APHA standards, each of a designated ADMI value. After a standard has been prepared,[9] the necessary data are acquired by means of a 400-700 nanometer scan. These data are reduced to percent transmittance values at integer nanometer increments. From these data, tristimulus valves are computed using the 30 Selected Ordinate method. Munsell values are determined, and the DE number is computed. After all six standards have been analyzed, a new standards equation (ADMI=f(DE)) is computed. The new data are then stored if the user so designates.

By a similar procedure, any number of check standards may be run. The analyst prepares the check standard, and enters the ADMI value. The

UVVIS INTERMEDIATE CHLOROPHYLL COMPUTATION

(DATE/TIME: ##/##/## ##: ## X M)

ANALYST: ####################
STATION/STUDY: ####################
EXTRACTION METHOD: ####################
COMPUTATION METHOD: ####################
INSTRUMENT: ####################

SAMPLE ID: ########
SAMPLE TYPE: ########
EXTRACT VOLUME: ###.## ML
CELL PATH LENGTH: ##.## CM
DILUTION FACTOR: ###.###

SAMPLE RAW DATA	VOLUME (LITERS)	(BEFORE ACIDIFICATION) ABSORB AT ### NM	### NM	### NM	(AFTER) ABS. AT ### NM	### NM	### NM
REPL. 1	#.##E±##	-#.###	-#.###	-#.###	-#.###	-#.###	-#.###
REPL. 2	#.##E±##	-#.###	-#.###	-#.###	-#.###	-#.###	-#.###
REPL. 3	#.##E±##	-#.###	-#.###	-#.###	-#.###	-#.###	-#.###
REPL. 4	#.##E±##	-#.###	-#.###	-#.###	-#.###	-#.###	-#.###
REPL. 5	#.##E±##	-#.###	-#.###	-#.###	-#.###	-#.###	-#.###
MEAN	#.##E±##	-#.###	-#.###	-#.###	-#.###	-#.###	-#.###
ST. DV.	#.###E±##	-#.####	-#.####	-#.####	-#.####	-#.####	-#.####
%REL SD	---#.###	---#.###	---#.###	---#.###	---#.###	---#.###	---#.###

SAMPLE UNCORR.	CHL-A (MG/M^3)	CHL-B (MG/M^3)	CHL-C (MG/M^3)	TOTAL CHL (MG/M^3)	RATIO CHL-A/B	RATIO CHL-A/C
REPL. 1	----#.###	----#.###	----#.###	----#.###	---#. ##	---#.##
REPL. 2	----#.###	----#.###	----#.###	----#.###	---#. ##	---#.##
REPL. 3	----#.###	----#.###	----#.###	----#.###	---#. ##	---#.##
REPL. 4	----#.###	----#.###	----#.###	----#.###	---#. ##	---#.##
REPL. 5	----#.###	----#.###	----#.###	----#.###	---#. ##	---#.##
MEAN	----#.###	----#.###	----#.###	----#.###	---#. ##	---#.##
ST. DV.	###.####	###.####	###.####	###.####	##.###	##.###
%REL SD	---#.##	---#.##	---#.##	---#.##	---#.##	---#.##

SAMPLE CORR.	UNCOR/COR ABS RATIO	PHEO-A (MG/M^3)	CHL-A (MG/M^3)	TOTAL CHL (MG/M^3)	RATIO CHL-A/B	RATIO CHL-A/C
REPL. 1	----#.###	----#.###	----#.###	----#.###	---#.##	---#.##
REPL. 2	----#.###	----#.###	----#.###	----#.###	---#.##	---#.##
REPL. 3	----#.###	----#.###	----#.###	----#.###	---#.##	---#.##
REPL. 4	----#.###	----#.###	----#.###	----#.###	---#.##	---#.##
REPL. 5	----#.###	----#.###	----#.###	----#.###	---#.##	---#.##
MEAN	----#.###	----#.###	----#.###	----#.###	---#.##	---#.##
ST.DV.	###.####	###.####	###.####	###.####	##.###	##.###
%REL SD	---#.##	---#.##	---#.##	---#.##	---#.##	---#.##

REPLICATE	BIOMASS (G/M^3)	AUTOTROPHIC INDEX (SAMPLE UNACIDIFIED)	AUTOTROPHIC INDEX (SAMPLE ACIDIFIED)
REPL. 1	----#.###	----#.###	----#.###
REPL. 2	----#.###	----#.###	----#.###
REPL. 3	----#.###	----#.###	----#.###
REPL. 4	----#.###	----#.###	----#.###
REPL. 5	----#.###	----#.###	----#.###
MEAN	----#.###	----#.###	----#.###
STND DEV	####.###	####.###	####.###
% REL S.D.	---#.##	---#.##	---#.##

Figure 7. Intermediate chlorophyll report format for a sample.

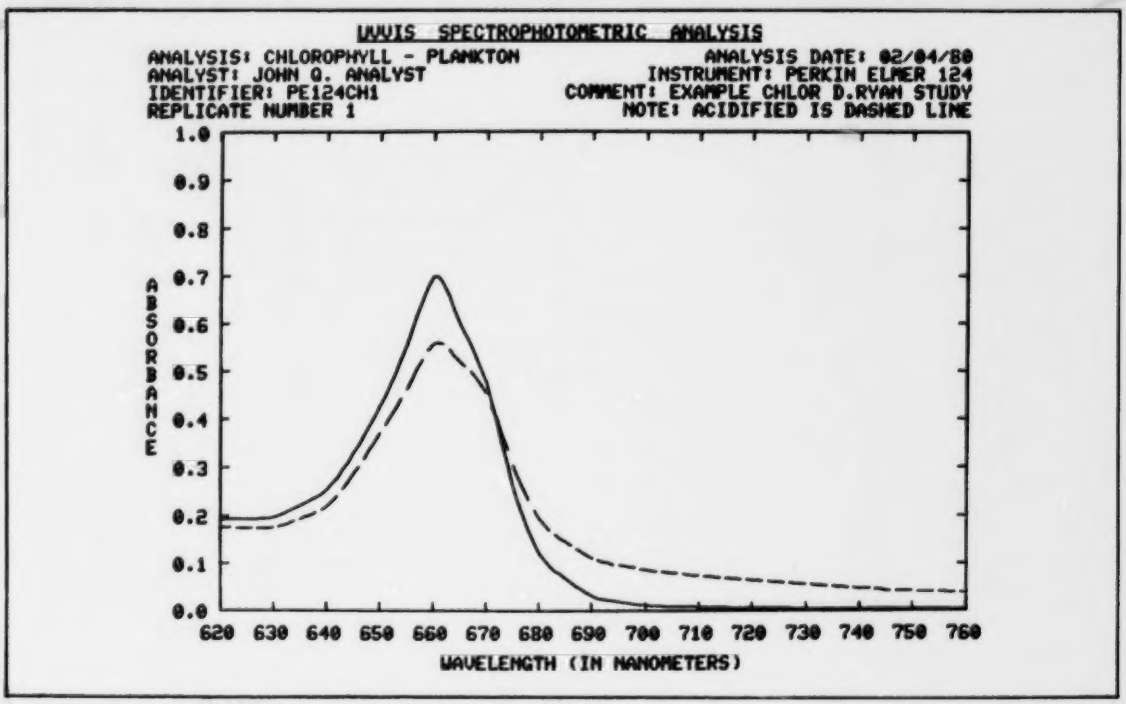


Figure 8. Example chlorophyll plot of a sample.

UVVIS CHLOROPHYLL COMPUTATION SUMMARY

DATE/TIME: MM/DD/YY HH:MM X. M.

ANALYST: ########################
STATION/STUDY: ####################
EXTRACTION METHOD: ####################
COMPUTATION METHOD: ####################
INSTRUMENT: ####################

DATE ANALYZED: ##/##/##
SAMPLE TYPE: ##########
EXTRACT VOLUME: ###. ## ML
CELL PATH LENGTH: ##. ## CM
COMMENT: ########################

(RAW DATA) SAMPLE ID NO.	SAMPLE VOLUME (LITERS)	(BEFORE ACIDIFICATION) ABSORB. AT ### NM	### NM	### NM	### NM	(AFTER) ABS. AT ### NM	### NM
########	#.##E±##	-#.###	-#.###	-#.###	-#.###	-#.###	-#.###
########	#.##E±##	-#.###	-#.###	-#.###	-#.###	-#.###	-#.###
########	#.##E±##	-#.###	-#.###	-#.###	-#.###	-#.###	-#.###

SAMPLE ID NO.	CHL-A (CORREC.) (MG/M^3)	PHEO-A (MG/M^3)	CHL-A (UNCORR.) (MG/M^3)	CHL-B (MG/M^3)	CHL-C (MG/M^3)	TOTAL CHLORO. (MG/M^3)
########	----#.###	----#.###	----#.###	----#.###	----#.###	----#.###
########	----#.###	----#.###	----#.###	----#.###	----#.###	----#.###
########	----#.###	----#.###	----#.###	----#.###	----#.###	----#.###

SAMPLE ID NO.	BIOMASS (G/M^3)	AUTOTROPHIC INDEX (SAMPLE UNACIDIFIED)	AUTOTROHPHIC INDEX (SAMPLE ACIDIFIED)
########	----#.###	----#.###	----#.###
########	----#.###	----#.###	----#.###
########	----#.###	----#.###	----#.###

Figure 9. Summary report format for chlorophyll analysis.

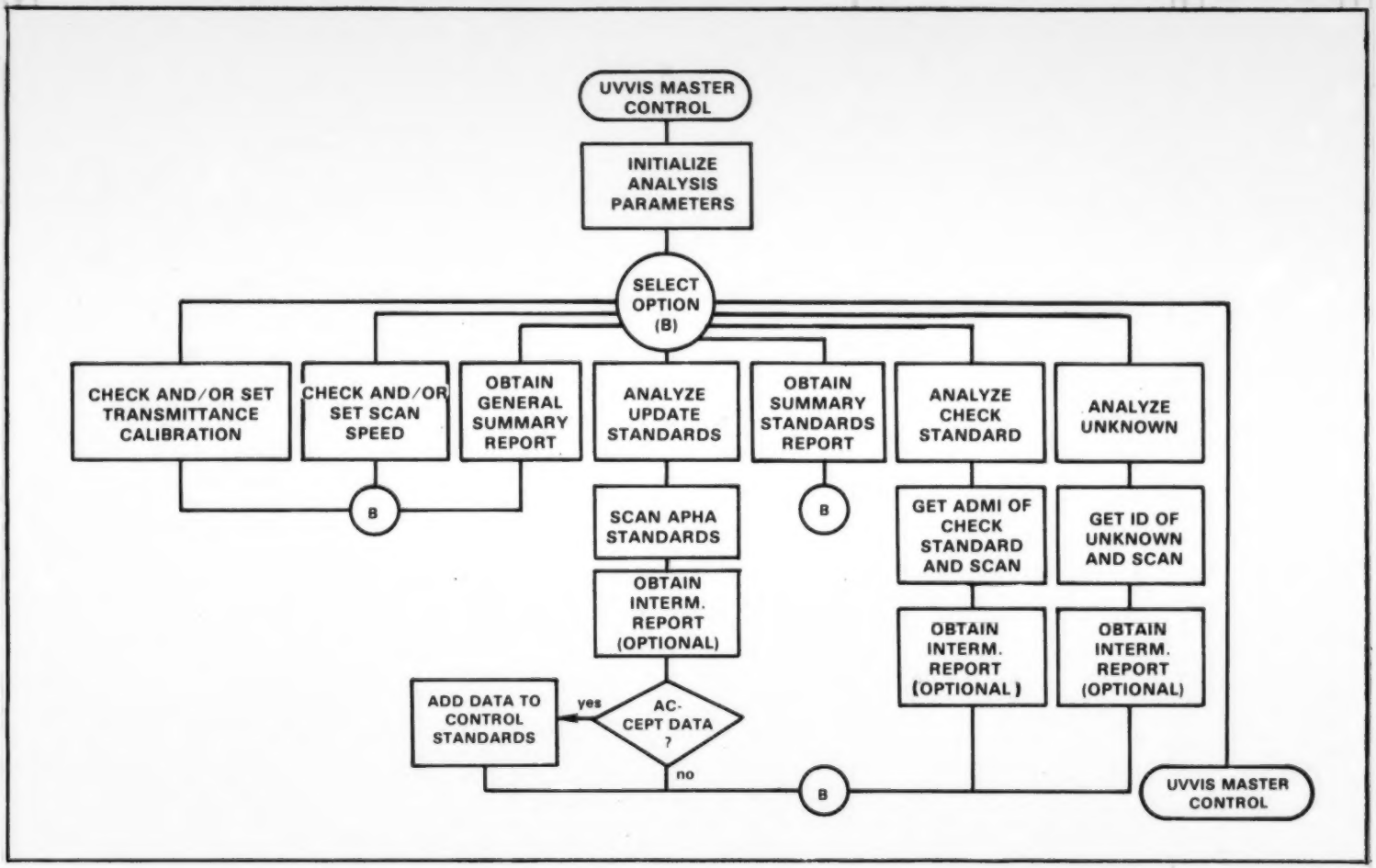


Figure 10. Features and command structure of the automated color analysis.

resultant DE and ADMI values are compared to standards data, and results are displayed on the teriminal. Any check standard may be used to update the historical standards data for the appropriate ADMI value.

Unknowns, that is, samples of unknown ADMI values, are analyzed by the same procedure as the standards and check standards. Any number of unknowns may be run, and check standards can be intermingled among samples at any point.

Intermediate terminal reports are issued after each update tandard, check standard, and unknown. Throughout the analysis session, general summary reports and a summary standards report may be obtained at the terminal and/or lineprinter.

5.6.2 Description of a Manual Run

For the most part, the procedure of a manual-input run follows that of an automated run, described above. Update standards, check standards, and unknowns may all be analyzed. The common three-filter tristimulus method (9) will be used. This method, as it would apply to the UVVIS system, is detailed in Appendix A.

5.6.3 Example Reports and Plots

Figures 11, 12, and 13 show intermediate report formats for an update standard, a check standard, and an unknown. A summary standards report format is exhibited in Figure 14 and the format of a general summary report is shown Figure 15. Figures 16, 17, and 18 show example plots of an update standard, a check standard, and an unknown.

5.6.4 Data Files

A standards file will maintain historical APHA standards data. It will contain the DE numbers, mean DE, standard deviation, and percent relative standard deviation for as many as six standards of the same ADMI value. There are six APHA standards with the ADMI values of 25, 50, 100, 150, 200, and 250 color units. There will be one standards file for automated run data, and another for manual run data.

Two summary files will be needed. A Data Summary File will contain all necessary scan results and an Output Report File will accumulate additional facts for the summary report.

Plot data will be maintained for each scan of an update standard, check standard, or unknown. After each scan, additional information, including some headings material, will be supplied to the plot reference file. (See section 5.7 below).

A permanent standards file will contain the constant factors used in the 30 Selected Ordinate Method.

UVVIS COLOR ANALYSIS
UPDATE STANDARDS REPORT

DATE/TIME: MM/DD/YY HH: MM X. M.

ANALYST: ##################### INSTRUMENT: #####################

STANDARDS CALIBRATION EQUATION ---
ADMI = -#.###E±## + (-#.###E±## * DE) + (-#.###E±## * DE^2)
(STANDARD ERROR OF ESTIMATE: ---#.###; %REL S.E.E.: --#.##)

ADMI OF APHA STANDARD	DE OF MOST RECENT UPDATE (A)	MEAN DE OF ALL STNDS. (B)	DE DIFFERENCE (A-B)=(D)	STANDARD DEV. OF MEAN DE	UPDATE CONTROL RATING
25	-#.####	-#.####	-#.####	#.####	###
50	-#.####	-#.####	-#.####	#.####	###
100	-#.####	-#.####	-#.####	#.####	###
150	-#.####	-#.####	-#.####	#.####	###
200	-#.####	-#.####	-#.####	#.####	###
250	-#.####	-#.####	-#.####	#.####	###

NOTE: CONTROL RATINGS BASED ON ABSOLUTE VALUE DE DIFF. (D') RELATIVE TO STND. DEV. (S) OF STANDARDS MEAN DE, SUCH THAT: * -- IN CONTROL (D'<2S), ** -- WARNING (2S<=D'<=3S); *** -- OUT OF CONTROL (D'>3S).

Figure 11. Intermediate color report format for update standards.

UVVIS COLOR ANALYSIS
CHECK STANDARDS REPORT

DATE/TIME: MM/DD/YY HH:MM X.M.

ANALYST: ##################### INSTRUMENT: #####################

CHECK STANDARD ID	APHA STND ADMI	MEAS'D DE (A)	MEAS'D ADMI (CALC)	STAND'S MEAN DE (B)	ST. DV. OF MEAN DE (C)	DE (A-B) (D)	CONTROL RATING (NOTE)
########	###	-#.####	-#.####	-#.####	#.####	-#.####	###
########	###	-#.####	-#.####	-#.####	#.####	-#.####	###
########	###	-#.####	-#.####	-#.####	#.####	-#.####	###
########	###	-#.####	-#.####	-#.####	#.####	-#.####	###
########	###	-#.####	-#.####	-#.####	#.####	-#.####	###
########	###	-#.####	-#.####	-#.####	#.####	-#.####	###

NOTE: CONTROL RATINGS BASED ON ABSOLUTE VALUE DE DIFF. (D') RELATIVE TO STND. DEV. (C) OF STANDARDS MEAN DE, SUCH THAT: * -- IN CONTROL (D' <2C); **-- WARNING (2C<=D'<=3C); ***-- OUT OF CONTROL (D'>3C).

Figure 12. Intermediate color report format for check standards.

UVVIS COLOR ANALYSIS
SAMPLE UNKNOWNS REPORT

DATE/TIME: MM/DD/YY HH:MM X.M.

ANALYST: ################## INSTRUMENT: ##################

SAMPLE ID	MEASURED DE NUMBER	CALCULATED ADMI VALUE	SAMPLE COMMENT
########	-#.####	---#.###	##############################
########	-#.####	---#.###	##############################
########	-#.####	---#.###	##############################
########	-#.####	---#.###	##############################
########	-#.####	---#.###	##############################
########	-#.####	---#.###	##############################

Figure 13. Intermediate color report format for unknowns.

UVVIS COLOR ANALYSIS
SUMMARY STANDARDS REPORT

DATE/TIME: MM/DD/YY HH: MM X. M.

ANALYST: ################## INSTRUMENT: ##################

STANDARDS CALIBRATION EQUATION ---
ADMI = -#.###E±## + (-#.###E±## * DE) + (-#.###E±## * DE^2)
(STANDARD ERROR OF ESTIMATE: ---#.####; %REL. S.E.: --#.##)

REPLICATE SETS OF STANDARDS (DATED) WITH STATISTICS	DE VALUES OF APHA STANDARDS ADMI 25	ADMI 50	ADMI 100	ADMI 150	ADMI 200	ADMI 250
REPL. 1 (MM/DD/YY)	-#.####	-#.####	-#.####	-#.####	-#.####	-#.####
REPL. 2 (MM/DD/YY)	-#.####	-#.####	-#.####	-#.####	-#.####	-#.####
REPL. 3 (MM/DD/YY)	-#.####	-#.####	-#.####	-#.####	-#.####	-#.####
REPL. 4 (MM/DD/YY)	-#.####	-#.####	-#.####	-#.####	-#.####	-#.####
REPL. 5 (MM/DD/YY)	-#.####	-#.####	-#.####	-#.####	-#.####	-#.####
REPL. 6 (MM/DD/YY)	-#.####	-#.####	-#.####	-#.####	-#.####	-#.####
REPLICATES MEAN	-#.####	-#.####	-#.####	-#.####	-#.####	-#.####
MEAN STAND. DEV.	#.####	#.####	#.####	#.####	#.####	#.####
% REL. STND DEV	---#.##	---#.##	---#.##	---#.##	---#.##	---#.##

Figure 14. Summary standards report format for color analysis.

UVVIS COLOR ANALYSIS
GENERAL SUMMARY REPORT

DATE/TIME: MM/DD/YY HH:MM X.M.

ANALYST: ##################### INSTRUMENT: #####################

STANDARDS INFORMATION:
CALIBRATION EQUATION ---
ADMI = -#.###E±## + (-#.###E±## * DE) + (-#.###E±## * DE^2)
(STANDARD ERROR OF ESTIMATE: ---#.###; %REL. S.E.E.: --#.##)
MOST RECENT STANDARDS UPDATE: MM/DD/YY

ADMI OF APHA STANDARD	MEAN DE OF ALL STANDARDS	STANDARD DEVIATION OF MEAN DE	% RELATIVE STND. DEV. OF MEAN DE
25	-#.####	#.####	---#.###
50	-#.####	#.####	---#.###
100	-#.####	#.####	---#.###
150	-#.####	#.####	---#.###
200	-#.####	#.####	---#.###
250	-#.####	#.####	---#.###

CHECK STANDARDS INFORMATION:

CHECK STANDARD ID	APHA STND ADMI	MEAS'D DE (A)	MEAS'D ADMI (CALC)	STAND'S MEAN DE (B)	ST. DV. OF MEAN DE (C)	DIFF. (A-B) (D)	CONTROL RATING (NOTE)
########	###	-#.####	---#.###	-#.####	#.####	-#.####	###
########	###	-#.####	---#.###	-#.####	#.####	-#.####	###
########	###	-#.####	---#.###	-#.####	#.####	-#.####	###
########	###	-#.####	---#.###	-#.####	#.####	-#.####	###
########	###	-#.####	---#.###	-#.####	#.####	-#.####	###
########	###	-#.####	---#.###	-#.####	#.####	-#.####	###

NOTE: CONTROL RATINGS BASED ON ABSOLUTE VALUE DE DIFF. (D') RELATIVE TO STND. DEV. (C) OF STANDARDS MEAN DE, SUCH THAT: * -- IN CONTROL (D' <2C); **-- WARNING (2C<=D'<=3C); ***-- OUT OF CONTROL (D'>3C).

UNKNOWNS INFORMATION:

SAMPLE ID	MEASURED DE NUMBER	CALCULATED ADMI VALUE	SAMPLE COMMENT
########	-#.####	---#.###	##############################
########	-#.####	---#.###	##############################
########	-#.####	---#.###	##############################
########	-#.####	---#.###	##############################
########	-#.####	---#.###	##############################
########	-#.####	---#.###	##############################

Figure 15. General summary report format for color analysis.

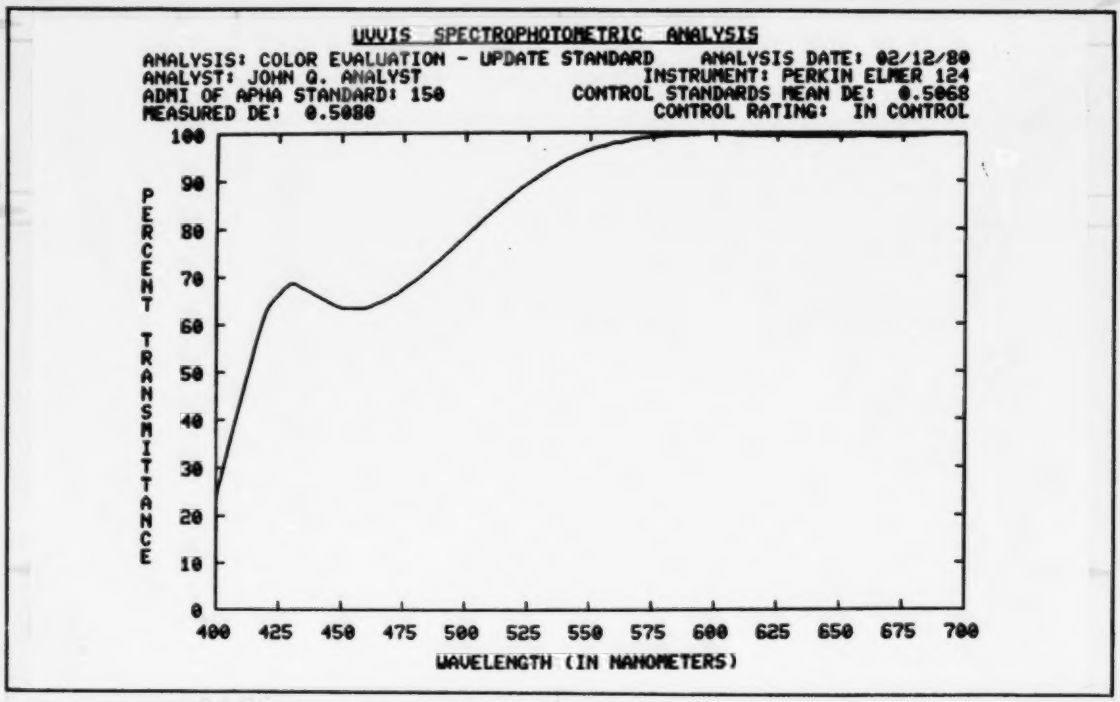


Figure 16. Example color plot of an update standard.

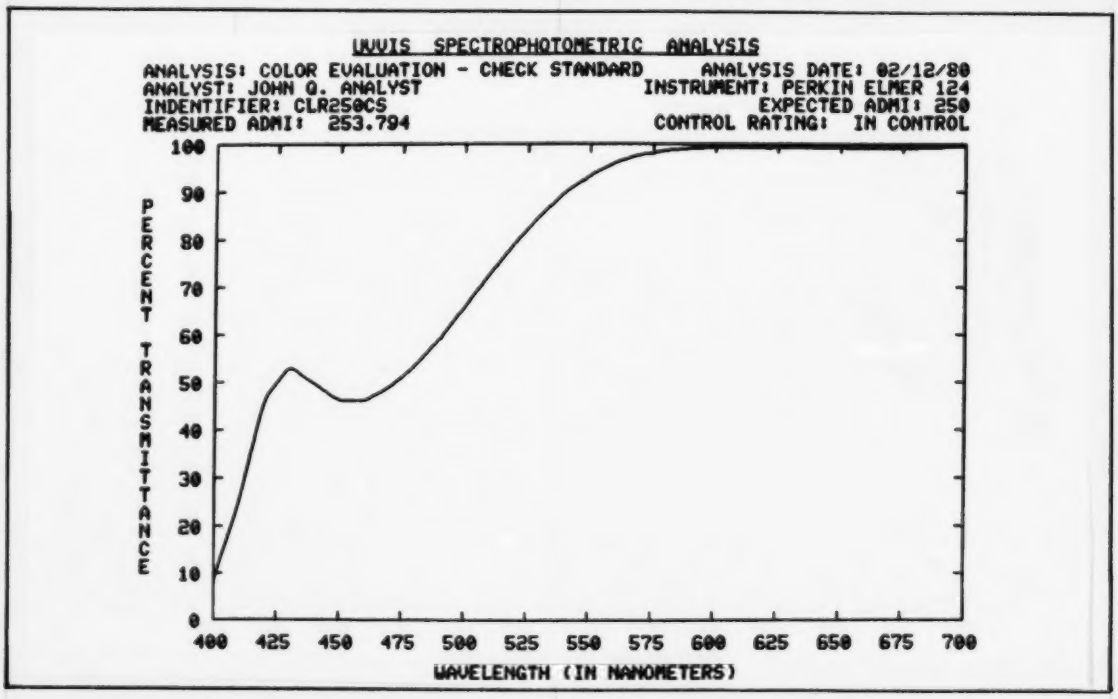


Figure 17. Example color plot of a check standard.

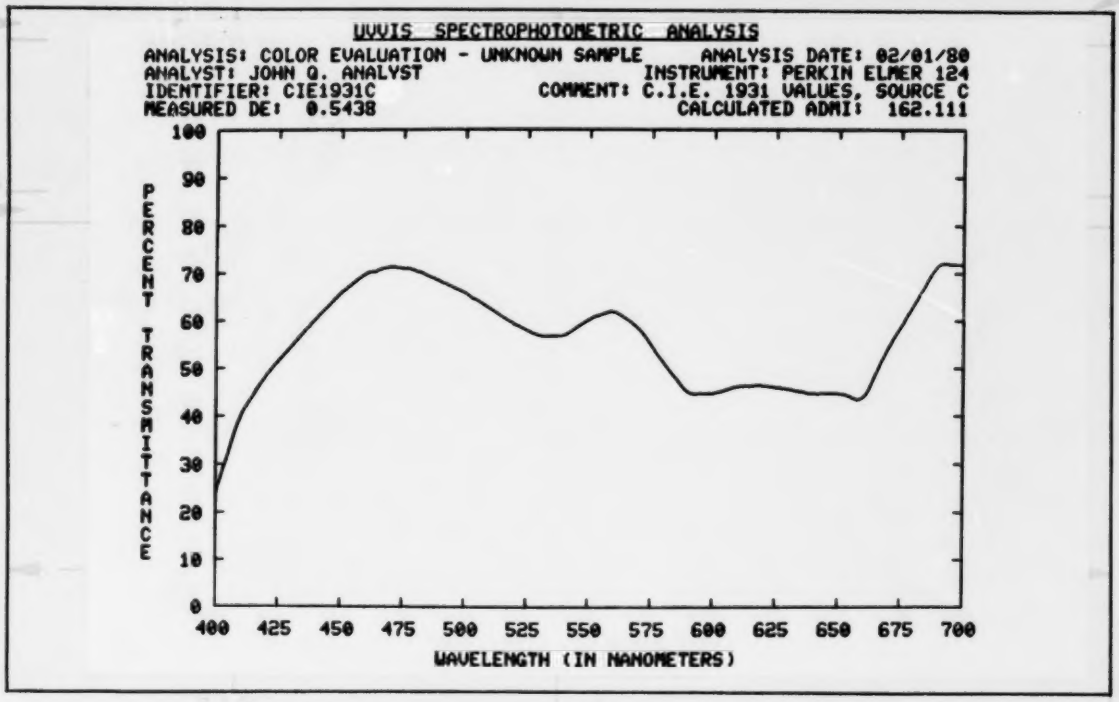


Figure 18. Example color plot of an unknown.

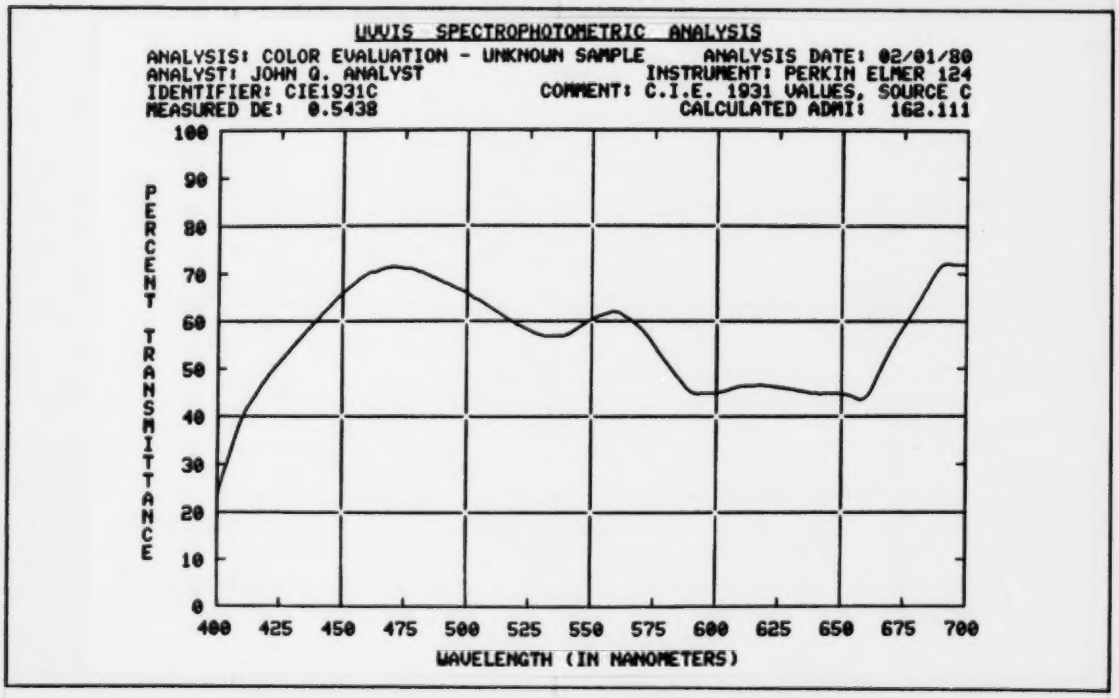


Figure 19. An example of the superimposed grid.

5.7 PLOTTING AND THE PLOT REFERENCE FILE

Formal plots can be made for Tektronix plotting terminals and for any software compatible plotting terminals.

Formal plots place wavelength on the horizontal axis and percent transmittance or absorbance on the vertical axis. For color data, wavelength ranges from 400 to 700 nanometers, and transmittance from 0% to 100%. For chlorophyll data, wavelength is from 620 to 760 nanometers, and absorbance from zero to one. If requested, a grid may be superimposed on the graph, as in Figure 19.

Informal plots are listed response curves which can be produced on the terminal or lineprinter. The users chooses one, two, five, or ten nanometer increments. For each increment, the wavelength and percent transmittance or absorbance values are listed. Along with each listed point is a star which is justified from the left according to its corresponding percent transmittance or absorbance value.

For each plot prepared, transmittance or absorbance data are stored in a plot data file. Any additional and heading information is stored in a plot reference file. The plot reference file includes the following information:

- analysis date
- analyst's name
- instrument type name
- headings
- lower and upper wavelength bounds
- number of scans (two for acidifed chlorophyll replicates)
- pointer(s) to initial data field in the plot data file

A distinct pair of plot files (data and reference) is produced for color analysis and for chlorophyll analysis.

SECTION 6

SIGNOFF SHEET

These documents, which describe a proposed automated spectrophotometer data system but do not constitute an implementation design, are approved by the undersigned interested parties with the understanding that changes in detail are likely and that implementation of all features depends upon the availability of funding and personpower.

Requestors:

_____ Curtis Ross, Director

_____ Warren Becker
EPA Region V Surveillance
and Analysis Division
Chicago, Illinois

_____ Orterio Villa, Director

_____ David C. Fleck
EPA Region III Surveillance
and Analysis Division
Annapolis, Maryland

_____ Cornelius I. Weber

_____ Nathan C. Malof
Environmental Monitoring
and Support Laboratory
Cincinnati, Ohio

Implementor:

_____ Gregory S. Roth
University of Cincinnati
Computing Center

Gas Chromatography System
Project Officer:
John M. Teuschler

_____

Laboratory Automation
Project Officer: _____ William L. Budde

REFERENCES

1. Budde, W. L., B. P. Almich and J. M. Teuschler. The Status of the EPA Laboratory Automation Project. EPA-600/4-77-025, U.S. Environmental Protection Agency, Cincinnati, Ohio, 1977.

2. Clark, C. T. and L. L. Schkade. Statistical Methods for Business Decisions. South-Western Publishing Company, Cincinnati, Ohio, 1969.

3. Catalogue of NBS Standards Reference Materials. National Bureau of Standards Special Publication 260. U.S. Department of Commerce, Washington, D.C., 1975, p. 55.

4. Keegan, H. J., J. C. Schleter and D. B. Judd. Glass Filters for Checking Performance of Spectrophotometer-Integrator Systems of Color Measurement. J. Res. of the National Bureau of Standards, 66A(3):203-221, 1962.

5. Weber, C. I. ed. Biological Field and Laboratory Methods for Measuring the Quality of Surface Waters and Effluents. EPA-670/4-3-001, U.S. Environmental Protection Agency, Cincinnati, Ohio, 1973.

6. Parsons, T. R. and J. D. H. Strickland. Discussion of Spectrophotometric Determination of Marine-Plant Pigments, with Revised Equations for Ascertaining Chlorophylls and Carotenoids. J. Marine Res., 21(3):155-163, 1963.

7. Jeffrey, S. W. and G. F. Humphrey. New Spectrophotometric Equations for Determining Chlorophylls a, b, c1, and c2 in Higher Plants, Algae and Natural Phytoplankton. Biochem. Physiol. Pflanzen, 167:191-194, 1975.

8. Almich, B. P. EPA Sample File Control Functional Design for the Nova Foreground. U.S. Environmental Protection Agency, Cincinnati, Ohio, unpublished document, 1978.

9. Office of Research and Development, Environmental Monitoring and Support Laboratory. Methods for Chemical Analysis of Water and Wastes. EPA-600/4-79-020, U.S. Environmental Protection Agency, Cincinnati, Ohio, 1979, 110.1:1-3.

10. Allen, W., et al. Determination of Color of Water and Wastewater By Means of ADMI Color Values. In: Proceedings of the 28th Industrial Waste Conference, Purdue University, Lafayette, Indiana, 1973, pp. 661-675.

11. American Public Health Association. Standard Methods for the Evaluation of Water and Wastewater, 14th ed. Washington, D.C., 1976, p. 68.

12. Rheinbolt, W. C. and J. P. Menard. Mechanized Conversion of Colorimetric Data to Munsell Renotations. J. Optical Soc. of America, 50(8):802-807, 1960.

13. McKlaren, K. Publications Sponsored by the Colour Measurement Committee-V. The Adams-Nickerson Colour-Difference Formula. J. Soc. Dyers Colour 86:354-366, 1970.

14. Savitsky, A. and M. J. E. Golay. Smoothing and Differentiation of Data by Simplified Least Squares Procedures. Analytical Chemistry, 36(8):1627-1639, 1964.

APPENDIX A

THREE-FILTER TRISTIMULUS METHOD OF MANUAL COLOR ANALYSIS

This method requires that measurements be made on a three-filter colorimeter. The method is also designed to use particular types of filters, as shown in Table 6.

A separate standards file is to be maintained for manual-input data, although its design is that of the standards file for the automated run. The file maintains up to six sets of replicate data for each of the APHA standards of 25, 50, 100, 150, 200, and 250 ADMI color units.

The steps of this computer-assisted three-filter tristimulus color method are as follows:

1. Prepare sample.[9]

2. Calibrate colorimeter with distilled water, setting transmittance at 100% level.

3. Measure sample and record percent transmittance for each filter.

4. Enter to computer percent transmittance for each filter.

5. Computer will make calculations of pseudo-tristimulus values, Munsell coefficients, DE numbers, and ADMI values (according to standards curve). The equations used to compute the pseudo-tristimulus values are:

$$X = (T_3 \times 0.1899) + (T_1 \times 0.791)$$

$$Y = T_2$$

$$Z = T_3 \times 1.1835$$

where T_1, T_2, and T_3 are the percent transmittance values for filters 1, 2, and 3 (see Table 6), respectively.

TABLE 6. FILTERS USED BY THE THREE-FILTER TRISTIMULUS METHOD

Filter Number	Filter Color	Corning Glass Works Designation Number	Wavelength (nm.) of Maximum Transmittance
1	Red	CS 3-107	590
2	Green	CS 4-98	540
3	Blue	CS 5-70	438